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EFFECT OF SELENIUM APPLICATION ON SOME OXIDOREDUCTIVE ENZYMES IN LOAMY SAND CONTAMINATED WITH DIESEL OIL

The aim of the study was to assess the impact of diesel oil on the activity of oxidoreductases in loamy sand, and to evaluate effect of selenium addition in the restoration of homeostasis of soil contaminated with diesel oil. The experiment was carried out under laboratory conditions with loamy sand. 0.05 mmol/kg of selenium (Se(IV) or Se(VI)), and diesel oil in the doses of 2, 10 and 50 g/kg were added to the soil samples. In soil treated with selenium and diesel oil activities of dehydrogenases, catalase, *o*-diphenol oxidase and nitrate reductase were measured on 1, 7, 14, 28, 56 and 112 day of the experiment. Contamination with diesel oil did not cause significant changes only in the activity of *o*-diphenol oxidase. The highest increase was observed in activity of dehydrogenases. Selenium limited hydrocarbons impact on activity of soil oxidoreductases, especially in loamy sand with diesel oil in the dose of 10 g/kg. Obtained results showed that this effect was reduced after treatment with selenium in both oxidation states but for nitrate reductase and catalase this reduction was reported after application of Se(VI) and Se(IV), respectively.

1. INTRODUCTION

Diesel oil (DO) is one of the most intensively used petroleum-based products. Petroleum compounds, introduced to the environment, affect both its abiotic and biotic elements. Soil contamination with hydrocarbons is caused by their intentional incorporation to ground and unintended leaking from storage tanks and pipelines or accidental spills [1]. Petroleum substances may have adverse effect on physical, chemical and biological properties of soil. These changes tend to upset the biological balance of soil. It usually changes the succession of microorganisms, which is directly associated with the

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activity of soil enzymes [2]. Enzymatic activity is a very good indicator of soil microbial population changes [3].

The most important soil enzymes belong to class of oxidoreductases and hydrolases. Oxidoreductases are the group of enzymes, which catalyze the transfer of electrons from donor to acceptor. The acceptor may be an organic and non-organic compound, as well as oxygen [4].

Microbial decomposition of petroleum and petroleum products is of considerable importance. Petroleum hydrocarbons are the rich source of carbon, and they are oxidized aerobically by a variety of microorganisms in soil. Maila and Cloete [5] showed 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 in soil, naturally occurring in plant residues. Additionally, polyphenol oxidases might incorporate aromatic xenobiotics into structure of humic substances [6]. Many microorganisms use nitrates as a source of terminal electron acceptors under anaerobic conditions and convert them to ammonia through a series of reactions. One of enzyme participating in these transformations is nitrate reductase (EC 1.6.6.1) [7].

Selenium is an important element, which has high antioxidant activity. Many authors reported changes in soil oxidoreductase activity after selenium treatment [8–10]. Selenium occurs naturally in the environment but with different levels. Selenium content in soils is most often in the range of 0.1–2 mg/kg, and its average content is 0.33 mg/kg. In Poland, the content of Se in soils is lower than the global average [11].

The aim of the study was to determine the oxidoreductase activity in loamy sand contaminated with diesel oil in dependence on selenium presence.

2. MATERIALS AND METHODS

Soil samples were collected from the Agricultural Experimental Station in Lipnik (53°24′ N and 14°28′ E), located in West Pomeranian Voivodeship, Poland. This field remained under conventional farming practices. The soil was classified as loamy sand. Some of its physical and chemical characteristics are as follows: pH_{KCl} 6.36, organic C 8.71 g/kg, total N 0.89 g/kg, total exchangeable bases (TEB) 10.50 cmol(+)/kg, cation exchange capacity (CEC) 13.02 cmol(+)/kg. To consider the spatial heterogeneity, soil samples were taken in triplicates, and each replicate consisted of five 10 cm auger cores. The soil samples were gently manually crumbled, mixed thoroughly, air dried at room temperature, and passed through a sieve with 2 mm mesh to remove stones and plant roots before being used. The soil material was divided into 0.5 kg samples. Selenium (Se(IV) or Se(VI)) in the amount of 0.05 mmol/kg, as aqueous solutions of H_2SeO_3 or H_2SeO_4, and diesel oil (purchased in Statoil petroleum station) in the doses of 2, 10 and

50 g/kg were added to the soil samples in appropriate combinations. Samples were adjusted to 60% maximum capillary water capacity, and they were incubated for eight weeks in tightly closed glass containers at 20 °C. Every treatment was replicated three times at each sampling stage.

In soil treated with selenium and diesel oil, oxidoreductase activities were measured on 1, 7, 14, 28, 56 and 112 day. The dehydrogenase activity was determined by the method described by Thalmann [12]. Soil samples were suspended in a 2,3,5-triphenyltetrazolium chloride solution and incubated at 25 °C. The 2,3,5-triphenylformazan (TPF) produced was extracted with acetone and measured photometrically at 546 nm. The *o*-diphenol oxidase activity was determined according to procedure presented by Perucci et al. [4]. It is based on a spectrophotometric determination (525 nm) of a red compound 4-(N-proline)-o-benzoquinone developed from enzymatic oxidation of catechol in the presence of proline. The nitrate reductase was determined as described by Abdelmagid and Tabatabai [13], using KNO_3 as a substrate. Soil samples were incubated at 25 °C under waterlogged conditions in an enclosed test tube. After incubation, released NO_2^- was extracted with KCl solution, and determined calorimetrically at 520 nm. Catalase activity was measured by the Johnson and Temple [14] method. The method involves titrating the hydrogen peroxide remaining in the soil after the incubation (not decomposed by the catalase in the soil sample) with potassium manganite(VII) in acidic conditions.

All analyses were done in three repetitions. Obtained data were submitted to three-way ANOVA in which diesel oil dose, selenium oxidation state and day of experiment were selected as factors. Tukey's test was used to identify possible differences between selenium application and diesel oil dose when the ANOVA was significant. The statistics were done using Statistica 10.0 (StatSoft).

3. RESULTS AND DISCUSSION

Treatments with diesel oil caused mainly increase in activity of dehydrogenases, but this effect depended on day of experiment and diesel oil dose. This stimulation in loamy sand with 2, 10 and 50 g/kg of diesel oil was observed for 14 (162–279% of control), 28 (207–677% of control) and 112 (109–788% of control) days (Fig. 1). Earlier reports indicated increased activity of dehydrogenases in soil polluted with petroleum hydrocarbons [15–17]. A three-way ANOVA on activity of dehydrogenases showed a significant difference at $P < 0.01$ for diesel oil dose and day of experiment. Selenium application did not cause a significant effect. However, two and three way interactions between diesel oil dose, selenium application and day of experiment were significant at $P < 0.01$ (Table 1). Tukey's test showed no significant effect of selenium (in both oxidation states) application on the mean activity of dehydrogenases in the soil uncontam-

inated with hydrocarbons and in soil with 2 g/kg of diesel oil. Compared with soil contaminated with 10 g/kg of diesel oil, significant decrease in activity of dehydrogenases was reported after application of selenium in both oxidation states.

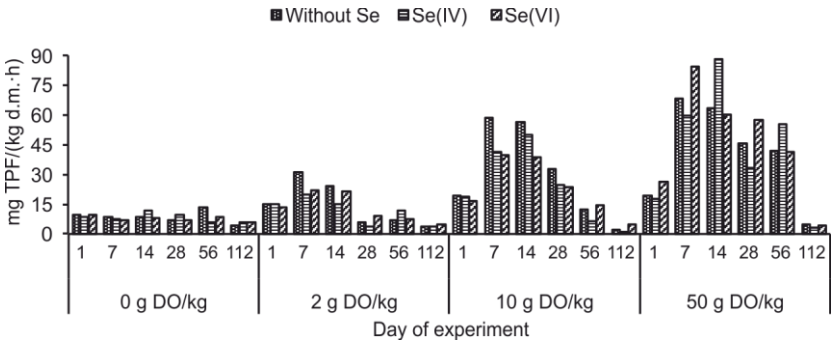

Fig. 1. Activity of dehydrogenases in loamy sand treated with various doses of diesel oil (DO) and selenium

Table 1

F-statistics of three-way ANOVA of the effects of diesel oil dose, selenium application, and day of experiment on the activity of dehydrogenases in loamy sand

Source of variation	Degrees of freedom *DF*	Mean square *MS*	*F* value[a]
Diesel oil dose (*DO*)	3	13156.14	427.62
Selenium application (*Se*)	2	67.70	2.20
Day of experiment (*DE*)	5	6024.87	195.83
DO×*Se*	6	129.58	4.21
DO×*DE*	15	1374.01	44.66
Se×*DE*	10	128.56	4.18
DO×*Se*×*DE*	30	121.72	3.96

[a]$P < 0.01$.

However, in soil containing 50 g/kg of diesel oil, significant increase in enzyme activity was observed after application of Se(VI) (Table 2). Previous research has shown that addition of selenium into soil contaminated with gasoline eliminated impact of hydrocarbons on soil dehydrogenases and peroxidases [10]. Among all enzymes in the soil environment, dehydrogenases belong to the most important ones. They are used indicators of overall soil microbial activity [18] because they occur intracellular in all living microbial cells [19]. Moreover, they are tightly linked with microbial oxidoreduction processes and they do not accumulate extracellular in the soil [20].

Table 2

Mean activities of dehydrogenases [mg TPF/(kg d.m.·h)]
in loamy sand treated with selenium versus diesel oil doses

Selenium application	Diesel oil dose [g/kg]			
	0	2	10	50
Without Se	8.59a	14.54a	30.43b	40.70a
Se(IV)	8.28a	11.57a	23.72a	42.98a
Se(VI)	7.80a	13.00a	23.12a	45.80b

Values denoted by the same letters within a column do not differ statistically (Tukey's test at $P < 0.05$).

Nitrate reductase activity in loamy sand with 2 g/kg of diesel oil was decreased on 14 and 28 day (68% and 44% of control, respectively). Its activity in soil contaminated with 10 and 50 g/kg of diesel oil was higher than control on 1, 7, 14, 56 and 112 day (137–198% of control and 118–147% of control, respectively) (Fig. 2).

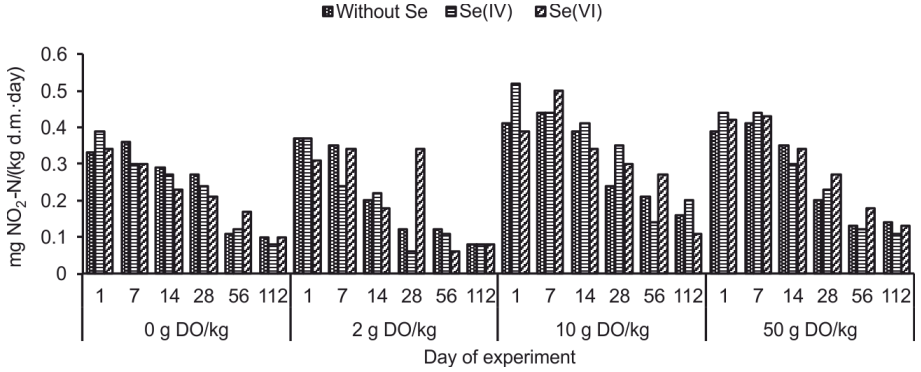

Fig. 2. Activity of nitrate reductase in loamy sand
treated with various doses of diesel oil (DO) and selenium

Ukiwe et al. [21] reported that petroleum products could inhibit nitrate reduction in soil. Furthermore, Acuna et al. [22] have shown that nitrogen deficiency in soils produced a decrease in the microbial biomass, which might have reduced hydrocarbon biodegradation. The three-way ANOVA for diesel oil dose, selenium application and day of experiment as the parameter showed significant effect for each factor and also two and three way interactions between the factors at $P < 0.01$, except selenium for which a significant effect occurred at $P < 0.05$ Three way interaction was significant at $P < 0.01$ (Table 3). According to Tukey's test, application of Se(VI) caused significant increase in nitrate reductase activity in soil contaminated with 10 g/kg of diesel oil, whereas in soil containing 50 g/kg of diesel oil, significant increase in this enzyme activity was reported after treating with Se(IV) (Table 4).

Table 3

F-statistics of three-way ANOVA of the effects of diesel oil dose, selenium application, and day of experiment on the activity of nitrate reductase in loamy sand

Source of variation	Degrees of freedom *DF*	Mean square *MS*	*F* value
Diesel oil dose (*DO*)	3	0.15	191.27[a]
Selenium application (*Se*)	2	0.01	4.33[b]
Day of experiment (*DE*)	5	0.45	565.15[a]
DO×*Se*	6	0.02	8.21[a]
DO×*DE*	15	0.02	8.75[a]
Se×*DE*	10	0.02	13.05[a]
DO×*Se*×*DE*	30	0.02	7.9[a]
Error	144	0.01	–

[a]$P < 0.01$.
[b]$P < 0.05$.

Table 4

Mean activities of nitrate reductase [mg NO_2^--N/(kg d.m.·day)] in loamy sand treated with selenium versus diesel oil doses

Selenium application	Diesel oil dose [g/kg]			
	0	2	10	50
Without Se	0.23a	0.19a	0.31a	0.27a
Se(IV)	0.23a	0.20ab	0.35b	0.27a
Se(VI)	0.22a	0.21b	0,29a	0.29b

Values denoted by the same letters within a column do not differ statistically (Tukey's test at $P < 0.05$).

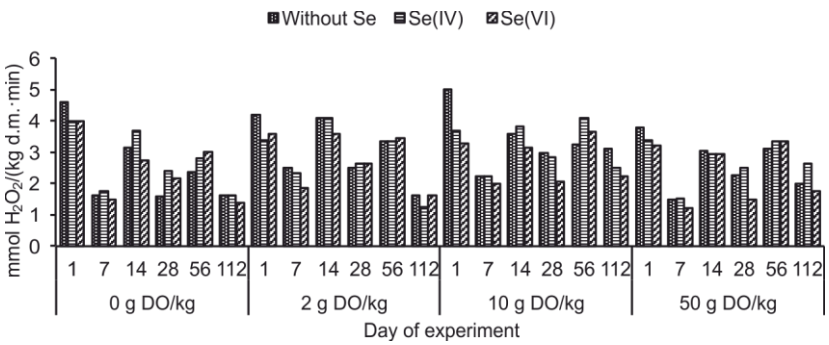

Fig. 3. Activity of catalase in loamy sand treated with various doses of diesel oil (DO) and selenium

Catalase activity in loamy sand was mainly stimulated by diesel oil. Application of 2 g/kg of diesel oil caused increase in catalase activity from day 7 to 56 (130–157% of control). After treatment with 10 g/kg of diesel oil, catalase activation was observed in all days of the experiment (109–193% of control), whereas contamination with 50 g/kg of diesel oil caused the increase in this enzyme activity only from day 28 to day 112 (123–142% of control) (Fig. 3). Many authors reported the increase [10, 17] or decrease [18, 23] in catalase activity in soil contaminated with petroleum hydrocarbons. Catalase is used in enzymatic protection against reactive oxygen species. It catalyzes decomposition of toxic hydrogen peroxide into oxygen and water [23].

Table 5

F-statistics of three-way ANOVA of the effects of diesel oil dose, selenium application and day of experiment on activity of catalase in loamy sand

Source of variation	Degrees of freedom *DF*	Mean square *MS*	*F* value
Diesel oil dose (*DO*)	3	3.64	30.70[a]
Selenium application (*Se*)	2	1.67	14.17[a]
Day of experiment (*DE*)	5	24.67	211.51[a]
DO×*Se*	6	0.41	3.50[a]
DO×*DE*	15	1.00	8.46[a]
Se×*DE*	10	0.72	6.14[a]
DO×*Se*×*DE*	30	0.17	1.42
Error	144	0.12	–

[a]$P < 0.01$.

Table 6

Mean activity of catalase [mmol H_2O_2/(kg d.m.·min)] in loamy sand treated with selenium versus diesel oil dose

Selenium application	Diesel oil dose [g/kg]			
	0	2	10	50
Without Se	2.48a	3.05a	3.36b	2.63ab
Se(IV)	2.71a	2.84a	3.20ab	2.72b
Se(VI)	2.47a	2.79a	2.74b	2.44a

Values denoted by the same letters within a column do not differ statistically (Tukey's test at $P < 0.05$).

In soil, catalase activity is closely related to the amount of aerobic microorganisms playing an important role in biodegradation of petroleum contaminants [24]. The three-way ANOVA for the diesel oil dose, selenium application and day of experiment as the

parameter showed a significant effect at $P < 0.01$ for each factor and also two way interactions between the factors. Three way interaction was not significant (Table 5). Tukey's test showed that only application of Se(VI) into soil with 10 g/kg of diesel oil caused decrease in mean activity of catalase (Table 6).

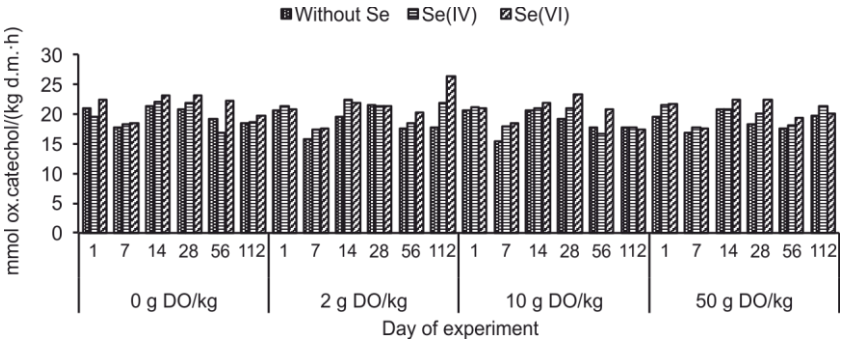

Fig. 4. Activity of *o*-diphenol oxidase in loamy sand treated with various doses of diesel oil (DO) and selenium

Table 7

F-statistics of three-way ANOVA of the effects of diesel oil dose, selenium application and day of experiment on the activity of *o*-diphenol oxidase in loamy sand

Source of variation	*DF* (degree of freedom)	*MS* (mean square)	*F* value
Diesel oil dose (*DO*)	3	0.24	2.84[a]
Selenium application (*Se*)	2	1.98	23.31[b]
Day of experiment (*DE*)	5	2.54	29.54[b]
DO×*Se*	6	0.09	1.06
DO×*DE*	15	0.19	2.30[b]
Se×*DE*	10	0.12	1.38
DO×*Se*×*DE*	30	0.12	1.45
Error	144	0.08	–

[a]$P < 0.05$.
[b]$P < 0.01$.

Activity of *o*-diphenol oxidase was slightly changed by addition of diesel oil in all doses (Fig. 4). These changes (increase or decrease) did not exceed 10%, compared with control. A three-way ANOVA on activity of *o*-diphenol oxidase showed a significant difference at $P < 0.05$ for diesel oil dose, and at $P < 0.01$ for selenium application and day of experiment. Interactions between the diesel oil dose and day of experiment were only significant at $P < 0.01$ (Table 7). The interaction between the diesel oil dose and selenium application was not significant. It was confirmed by Tukey's test (Table 8).

Similar effect was observed in previous studies on selenium effect in soil contaminated with gasoline and spent engine oil [10, 25].

Table 8

Mean activity of *o*-diphenol oxidase [mmol oxidized catechol/(kg d.m.·h)] in loamy sand treated with selenium versus diesel oil dose

Selenium application	Diesel oil dose [g/kg]			
	0	2	10	50
Without Se	21.01a	18.84a	18.60a	18.84a
Se(IV)	17.82a	20.52a	19.26a	19.98a
Se(VI)	15.36a	21.42a	20.46a	20.64a

Values denoted by the same letters within a column do not differ statistically (Tukey's test at $P < 0.05$).

4. CONCLUSIONS

- Contamination with diesel oil caused significant changes in activity of dehydrogenases, nitrate reductase and catalase in loamy sand. The highest increase was observed in activity of dehydrogenases.
- Application of selenium limited impact of hydrocarbons on the activity of soil oxidoreductases, especially in loamy sand with diesel oil in the dose of 10 g/kg.
- Obtained results showed that effect of diesel oil in the dose of 10 g/kg on activity of dehydrogenases was reduced after treatment with selenium in both oxidation states but for nitrate reductase and catalase this reduction was reported only after application of Se(VI) and Se(IV).

REFERENCES

[1] LIU P.W.G., CHANG T.C., WHANG L.-M., KAO C.-H., PAN P.-T., CHEN S.-S., *Bioremediation of petroleum hydrocarbon contaminated soil. Effects of strategies and microbial community shift*, Int. Biodeter. Biodegr., 2011, 65, 1119.

[2] ABOSEDE E.E., *Effect of crude oil pollution on some soil physical properties*, IOSR J. Agric. Vet. Sci., 2013, 6 (3), 14.

[3] ALRUMMAN S.A., STANDING D.B., PATON G.I., *Effects of hydrocarbon contamination on soil microbial community and enzyme activity*, J. King Saund. Univ. Sci., 2015, 27 (1), 31.

[4] PERUCCI R., CASUCCI C., DUMONET S., *An improved method to evaluate the o-diphenol oxidase activity of soil*, Soil Biol. Biochem., 2000, 32 (8–9), 1927.

[5] MAILA M.P., CLOETE T.E., *The use of biological activities to monitor the removal of fuel contaminants. Perspective for monitoring hydrocarbon contamination. A review*, Int. Biodeter. Biodegr., 2005, 55 (1), 1.

[6] SINSABAUGH R.L., *Phenol oxidase, peroxidase and organic matter dynamics of soil*, Soil Biol. Biochem., 2010, 42, 391.

[7] ZIÓŁKOWSKA A., WYSZKOWSKI M., *Toxicity of petroleum substances to microorganisms and plants*, Ecol. Chem. Eng. S, 2010, 17 (1), 73.
[8] NOWAK J., KĄKLEWSKI K., LIGOCKI M., *Influence of selenium on oxidoreductive enzymes activity in soil and in plants*, Soil Biol. Biochem., 2004, 36, 1553.
[9] BOROWSKA K., KOPER J., KOZIK K., RUTKOWSKA A., *Effect of slurry fertilization on the selenium content and catalase activity in lessive soil*, J. Elem., 2014, 19 (3), 649.
[10] STRĘK M., TELESIŃSKI A., *Change in oxidoreductase activity of selected microbial enzymes in gasoline-contaminated light soil in presence of selenium*, Ochr. Środ., 2015, 37 (1), 43 (in Polish).
[11] PATLORCZYK-PYTLIK P., KULCZYCKI G., *Content of selenium in arable soil near Wroclaw*, J. Elem., 2009, 14 (4), 755.
[12] THALMANN A., *Zur Methodik der Bestimmung der Dehydrogenaseaktivität im Boden mittels Triphenyltetrazoliumchlorid (TTC)*, Landwirt. Forsch., 1968, 21, 249 (in German).
[13] ABDELMAGID H.M., TABATABAI M.A. *Nitrate reductase activity of soils*, Soil Biol. Biochem., 1987, 19, 421.
[14] JOHNSON J.I., TEMPLE K.L., *Some variables affecting measurement of catalase activity in soil*, Soil Sci. Soc. Am. Proc., 1964, 28, 207.
[15] HAWROT M., NOWAK A., KŁÓDKA D., *Changes of dehydrogenases activity in soils polluted with diesel fuel*, Pol. J. Microbiol., 2005, 54, 1, 49.
[16] ACHUBA F.I., PERETIEMO C.B.O., *Effect of spent engine oil on soil catalase and dehydrogenase activities*, Int. Agrophysics, 2008, 22, 1.
[17] ACHUBA F.I., OKOH P.N., *Effect of petroleum products on soil catalase and dehydrogenase activities*, Open J. Soil Sci., 2014, 4, 399.
[18] SALAZAR S., SANCHEZ L., ALVAREZ J., VALVERDE A., GALINDO P., IGUAL J. PEIX A., SANTAREGINA I., *Correlation among soil enzyme activities under different forest system management practices*, Ecol. Eng., 2011, 37, 1123.
[19] YUAN B., YUE D., *Soil microbial and enzymatic activities across a chronosequence of Chinese pine plantation development on The Loess Plateau of China*, Pedosphere, 2012, 22, 1.
[20] MOESKOPS B., BUCHAN D., SLEUTEL S., HERAWATY L., HUSEN E., SARASWATI R., SETYORINI D., DE NEVE S., *Soil microbial communities and activities under intensive organic and conventional vegetable farming in west java, Indonesia*, Appl. Soil Ecol., 2010, 45, 112.
[21] UKIWE L.N., EGEREONU U.U., NJOKU P.C., NWOKO C.I.A., ALLINOR J.I.,. *Polycyclic aromatic hydrocarbons degradation techniques: A review*, Int. J. Chem., 2013, 5 (4), 43.
[22] ACUNA A.J., PUCCI O.H., PUCCI G.N., *Effect of nitrogen deficiency in the biodegradation of aliphatic and aromatic hydrocarbons in Patagonian contaminated soil*, Int. J. Res. Rev. Appl. Sci., 2012, 11 (3), 470.
[23] JINGYAN L., JIANXIONG W., YING H., XIANYONG H., *Effects of cotton on several enzymatic activities of the petroleum contaminated soil. A laboratory experiment*, Environ. Prot.. Eng., 2013, 39 (1), 163.
[24] MARGESIN R., WALDER G., SCHINNER F., *The impact of hydrocarbon remediation (diesel oil and polycyclic aromatic hydrocarbons) on enzyme activities and microbial properties of soil*, Acta Biotechn., 2000, 20 (3–4), 313.
[25] STRĘK M., TELESIŃSKI A., *Comparison of selenite(IV) and selenate(VI) effect on some oxidoreductive enzymes in soil contaminated with spent engine oil*, Plant Soil Environ., 2016, 162 (4), 157.