Journal of Elementology ISSN 1644-2296

Symanowicz B., Kalembasa S., Niedbała M., Toczko M., Skwarek K. 2018. *Fertilisation of pea (Pisum sativum* L.*) with nitrogen and potassium and its effect on soil enzymatic activity*. J. Elem., 23(1): 57-67. DOI: 10.5601/jelem.2017.22.1.1395

ORIGINAL PAPER

FERTILISATION OF PEA (*PISUM SATIVUM* L.) WITH NITROGEN AND POTASSIUM AND ITS EFFECT ON SOIL ENZYMATIC ACTIVITY*

Barbara Symanowicz[1], Stanisław Kalembasa[1], Mateusz Niedbała[2], Martyna Toczko[1], Korneliusz Skwarek[1]

[1]Soil Science and Plant Nutrition Department
[2]Institute of Social Science and Security
University of Natural Sciences and Humanities in Siedlce

Abstract

This study examined changes in soil enzymatic activity caused by constant nitrogen fertilisation and varied potassium fertilisation in soil abundant with available phosphorus. Pea (*Pisum sativum* L.) was used as the test plant. A field experiment was carried out in 2010-2012, in a field located at a greenhouse facility owned by the University of Natural Sciences and Humanities in Siedlce. The experiment was completely randomized and carried out in four replications with the following mineral fertilisation: control object, N, NK_1, NK_2, NK_3, NK_4 and NK_5. Mineral fertilisation was applied in kg ha^{-1}: N – 20, K_1 – 41.5, K_2 – 83, K_3 – 124, K_4 – 166, K_5 – 207.5. Nitrogen was applied as ammonium nitrate in early spring, potassium – as 60% potassium salt split into two doses: I (to 124 kg ha^{-1}) – in early spring, II (above 124 kg ha^{-1}) – after the first date of soil sampling. The soil samples were collected from the Ap horizon (0-30 cm) of the rhizosphere in four times. The activity of urease decreased significantly with the increasing doses of potassium. The highest activity of dehydrogenases was found in soil fertilised with nitrogen at 20 kg ha^{-1} and potassium at 166 kg ha^{-1}. Varied potassium fertilisation did not significantly affect the level of acidic or alkaline phosphatase. The biochemical index of potential soil fertility, as affected by nitrogen fertilisation, was the highest with nitrogen supplied at 20 kg ha^{-1} and with potassium at 166 kg ha^{-1}.

Keywords: mineral fertilisation, biochemical index, enzymatic activity.

dr hab. inż. Barbara Symanowicz, prof. UPH, Soil Science and Plant Nutrition Department, University of Natural Sciences and Humanities in Siedlce, Prusa 14, 08-110 Siedlce, Poland, e-mail: barbara.symanowicz@uph.edu.pl

* Supported by the Polish Ministry of Science and Higher Education as part of the statutory activities of the Soil Science and Plant Nutrition Department University of Natural Sciences and Humanities in Siedlce.

INTRODUCTION

In recent years, there has been a growing interest in legumes of cultivated plants. The root rhizosphere of plants is characterised by an increased microbial activity. Soil microorganisms are the source of many soil enzymes, which catalyse the reactions occurring during the mineralisation of organic matter (Zhao et al. 2009). Soil enzymes play essential roles in soil processes such as nutrient cycling and energy transformation by catalysing numerous chemical, physical and biological reactions (Lemanowicz et al. 2009, Borowska, Koper 2011). According to Cheng, Zhiping (2007) and Symanowicz et al. (2014), species of grown crops as well as long-term fertilisation can result in microbial community shifts in soils. The type of fertilisation in plant cultivation affects enzymatic activity and consequently the potential viability of plants to grow (Kalembasa, Symanowicz 2012, Andrzejewska et al. 2016). According to Koper, Lemanowicz (2008), the activity of urease, dehydrogenases and phosphatases depends on organic and mineral fertilisation, soil pH, the time of soil sampling, cultivated plant species, but also on the degree of soil contamination with heavy metals (Kucharski et al. 2009, Wyszkowska et al. 2013, Kaczyńska et al. 2015).

Pea (*Pisum sativum* L.) is the main leguminous crop. Chemical and biochemical conditions in the soil are the chief factors affecting the quality and quantity of peas. Balanced mineral fertilisation should be correlated with the optimum soil activity. Soil enzymes play a critical role in catalysing reactions leading to organic matter decomposition, and serve as bioindicators of the biochemical and microbial soil activity (Koper et al. 2008). According to Xie et al. (2009), the activity of dehydrogenases is an indicator of soil quality and microbial activity. Complete fertilisation of pea is a poorly investigated issue, and no studies on the impact of mineral fertilisation on the enzymatic activity have been conducted yet. There is a particular need for studies into the effect of intensive potassium fertilisation on the level of enzyme activity in growing leguminous plants.

The objective of the study was to determine the impact of constant nitrogen and potassium fertilisation applied to pea on changes in the activity of selected soil enzymes and the level of biochemical soil fertility index.

MATERIAL AND METHODS

Soil characteristics

The soil samples for laboratory analyses were collected in 2012, from the humus horizon (Ap-loamy sand). The soil in which pea was grown was formed from loamy sand and its pH was neutral. The abundance of available phosphorus in soil, as determined with the Egner-Riehm's method (DL), was

very high, in contrast to available potassium, also measured with the the Egner-Riehm's method, which was low.

Experimental design

A field experiment was carried out in 2010-2012, in a field located at a greenhouse facility owned by the University of Natural Sciences and Humanities in Siedlce (52°17′N, 22°28′E). The experiment was performed in a completely randomised method with four replications, and included one factor - seven mineral fertilisation levels: control object (with no mineral fertilisation); N; NK_1; NK_2; NK_3; NK_4; NK_5. Mineral fertilisation was applied in kg ha^{-1}: N – 20, K_1 – 41.5, K_2 – 83; K_3 – 124; K_4 – 166, K_5 – 207.5. Nitrogen was applied as ammonium nitrate in early spring, potassium – as 60% potassium salt split into two doses: I (to 124 kg ha^{-1}) – in early spring, II (above 124 kg ha^{-1}) – after the first date of soil sampling. The experimental plots measured 3 m^2 each.

Determination of the activity of enzymes

The activity of soil enzymes was determined four times: June, July, August, September (in the first ten days of each month). Urease activity was determined colorimetrically following the incubation of soil with urea (aqueous solution) and addition of citrate buffer, according to a modified method of Alef, Nannipieri (1998). The activity of soil dehydrogenases in soil was determined colorimetrically with the method by Casida et al. (1964) and Wolińska et al. (2016), using TTC (2,3,5-triphenyl tetrazolium chloride) as a substrate reduced to TPF (triphenyl formazan) during incubation. All colorimetric compounds were determined with a spectrofotometr UV-VIS Lambda 25 (Perkin Elmer, Whaltham, USA). The activity of acid phosphatase and alkaline phosphatase was determined with a method consisting of the incubation of a reactive mixture containing soil and a substrate. The Page's method (1982), with disodium 4-nitrophenyl phosphate hexahydrate in modified universal buffer (MUB) as a substrate, was used for determination of acid phosphatase (AcP) activity (at pH 6.5) and for alkalic phosphatase (AlP) activity (at pH 11). The colour intensity (yellow) due to released p-nitrophenol was then measured spectrophotometrically. Organic carbon was determined with the oxidation-titrimetric method by Kalembasa (1991). In this method, soil and potassium dichromate and an acid mixture (sulphuric acid and phosphoric acid - 5:1) were added. Assuming the green colour was corrected, an indicator (N-phenyl antranilic acid) was added and the suspension in the flask was titrated with Mohr's salt.

Statistical analysis

Results from chemical determinations were subjected to statistical analysis using analysis of variance Statistica 12 PL (Statsoft, Inc. 2016) and significant differences determined by Tukey's test. The criterion for signifi-

cance was set at $p < 0.05$. In order to find the correlations between of organic carbon, yield and activity of soil enzymes (Ure, Deh, AcP, AlP). Assessment of soil fertility growth potential based upon a single enzyme may be biased and thus several enzymes were examined in present study. Potential biochemical index of soil fertility (Mw) was calculated to include activites of Ure, Deh, AcP, AlP and organic carbon content KUCHARSKI et al. (2009): $Mw = (AcP + AlP + Deh + Ure \cdot 10^{-1}) \cdot \%C$.

RESULTS AND DISCUSSION

The enzymatic reaction speed is mainly determined by the enzyme concentration involved in a reaction, substrate concentration, temperature, the presence of activators and inhibitors and pH (KOPER, LEMANOWICZ 2008). The soil in which pea was grown was characterised by the optimum chemical composition (ANDRZEJEWSKA et al. 2016) for growth and development of the plant (Table 1).

Statistical calculations revealed a significant effect of potassium fertilisation on the activity of urease in soil samples taken on four dates (Table 2). According to YANG et al. (2008) and ZHAO et al. (2009), urease activity predominantly depends on the type of mineral and organic fertilization. Also,

Table 1

Chemical characteristics of experimental soil

pH_{KCl}	pH_{CaCl_2}	N_{tot}	C_{org}	$N\text{-}NH_4$	$N\text{-}NO_3$	P*	K*	Mg*
		g kg^{-1}		mg kg^{-1}				
6.9	6.6	2.1	29.8	40	300	240	87	93

* available forms

Table 2

Activity of urease (Ure) in mg $N\text{-}NH_4^+$ kg^{-1} d.m. of soil h^{-1}

Mineral fertilisation	Date of sampling				Mean
	June	July	August	September	
Control	520.8	563.5	622.7	357.7	516.2
N	516.7^{b}	602.7^{ab}	438.7^{ab}	582.0^{ab}	535.0^{ab}
NK_1	511.7^{b}	533.3^{ab}	456.0^{ab}	513.6^{ab}	503.6^{b}
NK_2	537.5^{b}	395.0^{ab}	538.7^{ab}	603.1^{ab}	518.6^{b}
NK_3	362.5^{ab}	416.7^{ab}	482.4^{ab}	663.6^{ab}	481.3^{ab}
NK_4	356.7^{ab}	320.8^{ab}	481.9^{ab}	668.4^{ab}	456.9^{ab}
NK_5	437.5^{ab}	500.0^{ab}	372.7^{ab}	450.2^{ab}	440.1^{ab}
Mean	463.3	476.0	484.7	548.4	493.1
$LSD_{0.05}$	33.1	16.4	12.7	12.7	18.7

Explanations are in Material and Methods. The data in table are means ($n = 3$), ^{a} letters indicate significant differences only with respect to control, ^{b} letters indicate significant differences among mineral fertilisation levels.

previous studies of Kalembasa, Symanowicz (2012) showed an increase in the urease activity under the influence of fertilization with NPKMg.

The highest mean activity of urease was determined in soil fertilised with nitrogen at 20 kg·ha^{-1}. The increased doses of potassium (124, 166 and 207.5 kg·ha^{-1}) significantly decreased the activity of urease (by 17.2% on average). Also the study by Yang et al. (2008) indicated a reduced urease activity under the influence of mineral fertilisation. Potassium applied at a dose of 160 kg ha^{-1} in the cultivation of many years eastern galega influenced the increase of urease activity (Symanowicz et al. (2014).

The activity of the urease under study in the soil samples taken on consecutive dates increased steadily. The analyses showed that the potassium fertilisation reduced the activity of urease in soil in June, July and August. The activity of the enzyme under study in soil samples taken in September, after the test plant had been harvested, was significantly increased by nitrogen and potassium fertilisation compared to the soil collected from the control site. Significantly (1.86 times) higher activity compared to the control site was found in soil fertilised with nitrogen at 20 kg ha^{-1} and nitrogen and potassium at 20 kg ha^{-1}. The mean urease activity in the soil sampled was positively correlated with acid phosphatase (AcP) activity (AcP = 0.19 + 0.003Ure; r = 0.78). The study saw an increase in of urease activity in the soil at successive sampling dates with increasing organic carbon. Urease is sensitive to the changes of soil organic matter. The findings of this study were confirmed in the studies carried out by Sołek-Podwika, Ciarkowska (2008). The high urease activity in soil observed in this study indicates intensive mineralisation of nitrogen compounds and it is related to a high content of C_{org} and N_{tot}.

The activity of dehydrogenases is an intermediate indicator of the biomass of soil microorganisms and the level of dehydrogenase activity increases together with microbial counts and the rate of their metabolism. The significantly highest mean activity of dehydrogenases was determined in soil fertilised with nitrogen at 20 kg ha^{-1} and potassium at 166 kg ha^{-1} (Table 3).

Table 3

Activity of dehydrogenases (Deh) in cm^3 H_2 kg^{-1} d.m. of soil h^{-1}

Mineral fertilisation	Date of sampling				Mean
	June	July	August	September	
Control	55.96	35.38	49.87	73.43	53.66
N	68.23^{ab}	77.57^{ab}	68.45^{ab}	73.86	72.03^{ab}
NK_1	54.77^{b}	44.46^{ab}	50.77^{b}	55.44^{b}	51.36^{b}
NK_2	54.92^{b}	35.05^{b}	46.45^{b}	62.36^{b}	49.69^{b}
NK_3	40.73^{ab}	33.91^{b}	66.50^{ab}	71.66^{b}	53.20^{b}
NK_4	79.66^{ab}	71.09^{ab}	78.12^{ab}	90.90^{b}	79.94^{ab}
NK_5	66.20^{ab}	62.52^{ab}	66.86^{ab}	68.81^{b}	66.10^{ab}
Mean	60.07	51.43	61.01	70.93	60.86
$LSD_{0.05}$	2.38	6.05	5.09	19.56	8.27

Explanations as under Table 2.

Moreover, dehydrogenases activity was the highest in soil collected from the same site in June, August and September. Dehydrogenases are good indicators of the effect of excessive fertilisation on soil biotic elements. In studies by Kaczyńska et al. (2015) in soil fertilised with compost dehydrogenases activity was 16% higher, and in the case of urea – 72% lower than that of the non- fertilised soil. Unlike the results of chemical analyses, biological parameters reflect the effect of the environment on the food chain and the course of enzymatic reactions in soil. Rusek (2006) claims that the activity of dehydrogenases is a source of information on the total microbiological activity of soil and its contamination. Dehydrogenases are often used as the indicator of soil fertility and it also can denote the amount and activity of soil microbes Gil-Sotres et al. (2005). Hence higher Deh in OM (fertilization with organic manure) soil indicated that long-term application of composted straw was more beneficial to microbial biomass and activity than the application of NPK and no fertilization Xie et al. (2009). The effect of spent engine oil on the soil dehydrogenases activity was a progressive increase in the values obtained as the concentrations of the spent engine oil increased (Achuba, Peretiemo-Clarke 2008). Fertilization with manure resulted in an increase of dehydrogenases and catalase activities in soil with increasing doses of manure (Lemanowicz et al. 2009, Borowska, Koper 2011).

The optimum pH of soil for the activity of acid phosphatase is 4.0-6.5, and for alkaline phosphatase 9.0-11.0 (Bielińska 2005, Koper, Lemanowicz 2008, Kalembasa, Symanowicz 2012). The varied doses of potassium fertilisers used in this study differentiated the activity of acidic phosphatase in soil samples collected in June, July and September (Table 4). The highest activity of the enzyme was observed in soil samples collected at the control site in autumn (September), after pea had been harvested. Nitrogen fertilisation at 20 kg ha^{-1} and potassium at 124 kg ha^{-1} significantly reduced the activity of acidic phosphatase in soil compared to its activity in the control soil during

Table 4

Activity of acid (AcP) and alkaline (AlP) phosphatases in mmol PNP kg^{-1} d.m. of soil h^{-1}

Mineral fertilisation	Date of sampling								Mean	
	June		July		August		September			
	AcP	AlP	AcP	AlP	AcP	AlP	AcP	AlP	AcP	AlP
Control	0.33	0.43	0.29	0.41	0.34	0.39	0.42	0.44	0.34	0.42
N	0.37^{ab}	0.42	$0,37^{ab}$	0.41	0.33	0.44^{a}	0.40^{b}	0.43	0.37^{ab}	0.42
NK_1	0.35^{b}	0.44	0.38^{ab}	0.45	0.33	0.41	0.35^{ab}	0.43	0.35	0.43
NK_2	0.34^{b}	0.44	0.30^{b}	0.40	0.38	0.45^{a}	0.35^{ab}	0.45	0.34^{b}	0.43
NK_3	0.29^{ab}	0.43	0.32^{ab}	0.41	0.34	0.43	0.40^{b}	0.46	0.34^{b}	0.43
NK_4	0.35^{b}	0.43	0.25^{ab}	0.42	0.32	0.44^{a}	0.40^{b}	0.45	0.33^{b}	0.43
NK_5	0.32^{b}	0.42	0.32^{ab}	0.44	0.31	0,43	0.38^{ab}	0.45	0.33^{b}	0.43
Mean	0.34	0.43	0.37	0.42	0.34	0.43	0.39	0.44	0.36	0.43
$LSD_{0.05}$	0.04	n.s.	0.03	n.s.	n.s.	0.05	0.03	n.s.	0.03	n.s.

Explanations as under Table 2.

the growing season of the test plant (in June). The activity of AcP in soil increased in July and August to the level of 0.38 mmol PNP kg^{-1} d.m. of soil h^{-1} after fertilisation with NK_1 (N – 20, K – 41.5 kg ha^{-1}) and NK_2 (N – 20, K – 83 kg ha^{-1}). Varied potassium fertilisation did not significantly affect the alkaline phosphatase activity. It was at a similar level (0.42-0.43 mmol PNP kg^{-1} d.m. of soil h^{-1}). A significant increase in AIP activity (by 11.28% compared to the control site) was observed in August, in soil fertilised with N – 20 kg ha^{-1}; N – 20, K – 83 kg ha^{-1} and N – 20, K – 166 kg ha^{-1}. The highest activity of AcP and AIP was observed in soil samples collected one month after pea had been harvested (the first ten days of September). An increase in phosphatase activity was related to a decreased soil temp. to 15°C. As well as pH 6.33-7.28 observed in the experiment of Małachowska-Jutsz, Niesler (2015) was higher, which could have effected a decrease of acid phosphatase activity (0.678-0.402 mmol PNP kg^{-1} d.m. of soil h^{-1}), but in the studies by Koper, Lemanowicz (2008), Radulov et al. (2011), the highest amount of acid phosphatase (1.53 mmol PNP kg^{-1} d.m. of soil h^{-1}) was determined in soil with pH_{KCl} of 5.3-5.5 fertilised with N – 120 kg ha^{-1}. According to Bielińska (2005), an optimum pH for alkaline phosphatase is in the range of 8 - 10, and for the acid phosphatase 4 - 6.

The mean total organic carbon content in the soil was high: from 3.41 to 3.83% (Table 5). The results are significantly higher than those reported by Kucharski et al. (2009). However, the highest content of organic carbon (4.96%) was found in soil collected in September in the NK_3 site (N – 20, K – 124 kg ha^{-1}). This element originated from organic residues left after the pea was harvested. Statistical calculations revealed significant differences in the TOC content in soil samples collected in June between the NK_4 (N – 20, K – 166 kg ha^{-1}) and NK_5 (N – 20, K – 207.5 kg ha^{-1}) fertilisation sites. Significant differences in TOC in soil samples collected in July were also observed between the N (N – 20 kg ha^{-1}) and NK_3 (N – 20, K – 124 kg ha^{-1}) sites and

Table 5

Total organic carbon (TOC %) and biochemical index of soil fertility (Mw)

Mineral fertilisation	Date of sampling								Mean	
	June		July		August		September			
	TOC	Mw	TOC	Mw	TOC	Mw	TOC	Mw	TOC	Mw
Control	3.39	368.8	3.25	300.4	3.49	393.9	4.62	508.5	3.69	392.9
N	3.71	447.7	3.88^b	537.8	3.18	359.6	3.93	522.3	3.68	466.9
NK_1	3.79	404.5	3.73^b	367.8	3.68	357.4	4.13	444.3	3.83	393.5
NK_2	3.65	399.5	3.44	258.9	3.89	393.5	3.66	451.9	3.66	375.9
NK_3	3.20	248.6	2.74^b	209.1	3.50	404.3	4.96	689.8	3.60	387.7
NK_4	3.98^b	462.1	3.46	359.3	3.51	446.0	3.99	632.8	3.74	475.0
NK_5	2.78^b	307.7	3.49	395.3	3.01	315.7	4.36	499.9	3.41	379.7
Mean	3.50	377.0	3.43	346.9	3.47	381.5	4.24	535.5	3.66	410.2
$LSD_{0.05}$	0.95	-	0.82	-	n.s.	-	n.s.	-	n.s.	-

Explanations as under Table 2.

between NK_1 (N – 20, K – 41.5 kg ha^{-1}) and NK_3 (N – 20, K – 124 kg ha^{-1}). The content of carbon was positively correlated with the activity of urease.

The final study result is the biochemical index (M_w) of the soil (Table 5). The time point of soil sampling (soil temperature) had a decisive impact on the value of the biochemical soil fertility index. Moreover, a significant correlation was observed between the soil biochemical fertility index and the content of organic carbon in the soil (r_1 = 0.84, r_2 = 0.84, r_3 = 0.55, r_4 = 0.55). The index depended on the activity of the enzymes under study, the total organic carbon content in the soil under analysis, and nitrogen and potassium fertilisation. The high activity of urease and dehydrogenases and high levels of TOC in the soil under analysis resulted in the maximum values of the biochemical index. The mean biochemical index for the samples under analysis was the highest (475.0 and 466.9) for the soil samples collected from the NK_4 (N – 20, K – 166 kg ha^{-1}) and N (N – 20 kg ha^{-1}) sites. Calculations

Table 6

Simple correlation coefficients between enzyme activity and soil parameters (TOC, Mw)

Parameters	Ure	Deh	AcP	AlP	TOC
Date of sampling – June					
Ure	–				
Deh	n.s.	–			
AcP	0.50**	0.66**	–		
AlP	n.s.	n.s.	n.s.	–	
TOC	n.s.	n.s.	0.71**	0.47**	–
Mw	n.s.	0.71**	0.95*	n.s.	0.84*
Date of sampling – July					
Ure	–				
Deh	n.s.	–			
AcP	0.71**	n.s.	–		
AlP	n.s.	n.s.	n.s.	–	
TOC	0.43**	0.63**	0.44**	n.s.	–
Mw	0.55**	0.86*	0.45**	n.s.	0.84*
Date of sampling – August					
Ure	–				
Deh	-0.55**	–			
AcP	0.60**	-0.67**	–		
AlP	-0.45**	0.41**	n.s.	–	
TOC	0.60**	-0.59**	0.78*	n.s.	–
Mw	0.57**	n.s.	n.s.	n.s.	0.55**
Date of sampling – September					
Ure	–				
Deh	n.s.	–			
AcP	n.s.	0.72**	–		
AlP	n.s.	n.s.	n.s.	–	
TOC	n.s.	n.s.	0.55**	n.s.	–
Mw	0.57**	0.68**	0.60**	0.62**	0.55**

* $\alpha \leq 0.05$, ** $\alpha \leq 0.01$

revealed a diverse effect of fertilisation on the biochemical index on consecutive soil sampling dates. The highest biochemical index was calculated for the samples of soil collected in September, which was fertilised with nitrogen at 20 kg ha^{-1} and with potassium at 124 kg ha^{-1}. The results are significantly higher than those reported by Kalembasa, Symanowicz (2012), Kucharski et al. (2009) and Kucharski et al. (2011), who carried out the studies under laboratory conditions. The impossibility of comparing the results from field studies and laboratory experiments has been also indicated by other authors (Cheng, Zhiping 2007, Iovieno et al. 2009). Wyszkowska et al. (2013) recommended this indicator as one of the best for an evaluation of biochemical soil quality.

Values of correlation coefficients calculated during the statistical processing in most cases revealed significant dependencies between the factors studied in the experiment (Table 6). Following were high values of correlation coefficients achieved in three terms of soil sampling (June, July and August). High values of correlation coefficients were obtained between AcP vs. Mw, TOC vs. Mw (in June and July), Deh vs. Mw as well as AcP and TOC. The increasing activity of AcP in soil samples collected in June and Deh in soil samples taken in July raised the index of biochemical soil fertility (Mw = -564.04 + 2803.07AcP; r = 0.95 and Mw = 93.35 + 4.93Deh; r = 0.86). The increase in the carbon content of the analysed soil samples resulted in an increase of the soil fertility biochemical index (Mw) in June (Mw = -169.24 + 156.07 TOC; r = 0.84 and July (Mw = 495.71 + 245.88 TOC).

CONCLUSIONS

1. The analysis of soil enzymatic activity indicated high urease and dehydrogenases activity and low phosphatase activity.

2. The diverse potassium fertilisation, high abundance of available phosphorus in the soil and neutral soil pH all facilitated accelerated mineralisation of organic matter in soil and a reduction of urease activity.

3. Fertilisation with nitrogen supplied at 20 kg ha^{-1} and potassium at 166 kg ha^{-1} resulted in the maximum activity of dehydrogenases and the highest mean biochemical index of potential soil fertility.

REFERENCES

Achuba F.I., Peretiemo-Clarke B.O. 2008. *Effect of spent engine oil on soil catalase and dehydrogenase activities.* Int. Agrophys., 22(1): 1-4. www.international-agrophysics.org

Alef K., Nannipieri P. 1998. *Methods in Applied Soil Microbiology and Biochemistry.* Eds. Alef K., Nannipieri P. Academic Press. Harcourt Brace & Company. Publisher London, pp 576.

Andrzejewska J., Ignaczak S., Barzyk P. 2016. *Oil content and fatty acid profile in seeds of Polish breeding lines and cultivars of legumes.* Acta Sci. Pol. Agricult., 15(2): 3-13.

Bielińska E. J. 2005. *Methods of determination of phosphatase activity*. Acta Agroph., 3: 63-74.

Borowska K., Koper J. 2011. *Dynamics of changes of selenium content in soil and red clover (Trifolium pretense* L.*) affected by long-term organic fertilization on the background of selected soil oxidoreductases*. Pol. J. Environ. Stud., 20(6): 1403-1410.

Casida L.E. Jr., klein D.A., Santoro T. 1964. *Soil dehydrogenase activity*. Soil Sci., 98(6): 371-376.

Cheng H., Zhiping C. 2007. *Size and activity of the soil microbial biomass and soil enzyme activity in long-term field experiments*. Word J. Agric. Sci., 3(1): 63-70.

Gil-Sotres F., Trasar-Cepeda C., Leirós M. C., Seoane S. 2005. *Different approaches to evaluating soil quality using biochemical properties*. Soil Biol. Biochem., 37(5): 877-887. http://dx.doi.org/10.1016/j.soilbio.2004.10.003

Iovieno P., Morra L., Leone A., Pagano L., Alfani A. 2009. *Effect of organic and mineral fertilizers on soil respiration and enzyme activities of two Mediterranean horticultural soils*. Biol. Fertil. Soils, 45(5): 555-561. DOI: 10.1007/s00374-009- 0365-z

Kaczyńska G., Borowik A., Wyszkowska J. 2015. *Soil dehydrogenases as an indicator of contamination of the environment with petroleum products*. Water Air Soil Pollut., 226(11): 372-382. DOI 10.1007/s11270-015-2642-9

Kalembasa S. 1991. *Quick method of determination of organic carbon in soil*. Pol. J. Soil Sci., 24(1): 17-21.

Kalembasa S., Symanowicz B. 2012. *Enzymatic activity of soil after applying various waste organic materials, ash, and mineral fertilizers*. Pol. J. Environ. Stud., 21(6): 1635-1641.

Koper J., Lemanowicz J. 2008. *Effect of varied mineral nitrogen fertilization on changes in the content of phosphorus in soil and in plant and the activity of soil phosphatases*. Ecol. Chem. Eng. S., 15(5): 465-471.

Koper J., Piotrowska A., Siwik-Ziomek A. 2008. *Activity of dehydrogenases, invertase and rhodanase in forest rusty soil in the vicinity of the Anwil nitrogen plant in Włocławek*. Ecol. Chem. Eng. A., 15(3): 237-243.

Kucharski J., Boros E., Wyszkowska J. 2009. *Biochemical activity of nickel contaminated soil*. Pol. J. Environ. Stud., 18(6): 1039-1044.

Kucharski J., Wieczorek J., Wyszkowska J. 2011. *Changes in enzymatic activity in sandy loam sill exposed to zinc pressure*. J. Elem., 16(4): 577-589. DOI: 10.5601/jelem.2011.16.4.07

Lemanowicz J., Koper J., Igras J. 2009. *The dependence between farmyard manure and mineral nitrogen fertilization and some oxidoreductive enzymes in the winter wheat rhizosphere*. Adv. Agric. Sci. Probl., 537: 235-241.

Małachowska-Jutsz A., Niesler M. 2015. *The effect of calcium peroxide on the phenol oxidase and acid phosphatase activity and removal of fluoranthene from soil*. Water Air Soil Pollut., 226(11): 365-372. DOI 10.1007/s11270-015-2632-y

Page A.L. 1982. *Methods of Soil Analysis*. Part 2. *Chemical and microbiological properties*. Madison, WI, USA: American Society of Agronomy, Inc. Soil Science Society of America, Inc. Publishers. Burns R.G. Soil enzymes. Academic Press. New York.

Radulov I., Berbecea A., Sala F., Crista F., Lato A. 2011. *Mineral fertilization influence on soil pH, cationic exchange capacity and nutrient content*. Res. J. Agric. Sci., 43(3): 160-165.

Rusek A. 2006. *Dehydrogenase activity in soils polluted by diesel oil in a field lysimeter experiment*. Soil Sci. Ann., 57(3/4): 106-116. (in Polish)

Sołek-Podwika K., Ciarkowska K. 2008. *Urease activity in soils antropogenically enriched in sulphur compounds*. Soil Sci. Ann., 59(2): 196-200. (in Polish)

Statsoft, Inc. 2016. *Statistica* (data analysis software system), version 12.0.

Symanowicz B., Kalembasa S., Skorupka W., Niedbała M. 2014. *The changes of enzymatic activity of soil under eastern galega (Galega orientalis* Lam.*) after NPKCa fertilization*. Plant Soil Environ., 60(3): 123-128.

Wolińska A., Zapasek M., Stępniewska Z. 2016. *The optimal TTC dose and its chemical reduction level during soil dehydrogenase activity assay*. Acta Agroph., 23(2): 303-314.

Wyszkowska J., Borowik A., Kucharski M., Kucharski J. 2013. *Applicability of biochemical indices to quality assessment of soil polluted with heavy metals*. J. Elem., 18(4): 733-756. DOI: 10.5601/jelem.2013.18.4.504

Xie W., Zhou J., Wang H., Chen X., Lu J., Chen X. 2009. *Short-term effects of copper, cadmium and cypermethrin on dehydrogenase activity and microbial functional diversity in soils after long-term mineral or organic fertilization*. Agric. Ecosyst. Environ., 129(4): 450-456. http://dx.doi.org/10.1016/j.agee.2008.10.021

Yang L., Li T., Li F., Lemcoff J.H., Cohen S. 2008. *Fertilization regulates soil enzymatic activity and fertility dynamics in cucumber field*. Scientia Horticult., 116(1): 21-26. http://dx.doi.org/10.1016/j.scienta.2007.11.001

Zhao Y., Wang P., Li J., Chen Y., Ying X., Liu S. 2009. *The effect of two organic manures on soil properties and crop yields on a temperate calcareous soil under a wheat-maize cropping system*. Europ. J. Agron., 31(1): 36-42. http://dx.doi.org/10.1016/j.eja.2009.03.002