Original Research

Enzymatic Activity of Urease and Degydrogenase in Soil Fertilized With GWDA Compost with or without a PRP SOL Addition

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Abstract

In a two-year field experiment (2008-09), the effect of increasing doses of compost produced from municipal sewage sludge with or without the addition of active substance PRP®SOL (PRP Technologies, France) on urease and dehydrogenase activities during cultivation of winter wheat and spring rapeseed was studied. The experimental design included a control treatment with standard mineral fertilization and three levels of organic fertilization. The used compost doses were equivalent to 100, 200, and 300 kg N·ha^{-1}. Organic fertilization was carried out on 28 September 2007. In 2008 and 2009 the whole experimental area was fertilized with active substance PRP SOL at a 150 kg·ha^{-1} dose and multicomponent fertilize Polifoska 6 at a 200 kg·ha^{-1} dose. Due to the low nitrogen content in Polifoska 6 (6% N), urea top-dressing for winter wheat and spring rapeseed was applied at a dose of 100 kg N·ha^{-1} in two times periods. Soil samples for chemical analyses were collected from the arable layer (0-25 cm) under winter wheat four times: April, May, and July (soil sampling times I to III), and after its harvest at the end of August 2008 (sampling time IV). In spring 2009, spring rapeseed was sown in the same field, and soil samples for analyses were collected in the same times I to III (April, May, and July), while soil sampling time IV following the rapeseed harvest fell at the beginning of August 2009. It was found that fertilization with a triple dose of compost with a PRP SOL addition increased the soil pH_{KCl} value as compared to control soils. Higher organic carbon (OC), nitrogen (N), and phosphorus (P) contents were found in the soils collected from experimental plots being fertilized with a double and a triple dose of municipal sewage sludge compost with a PRP SOL addition. Significant increase in urease activity was found between soil sampling times I and IV (specify the treatment here). The applied fertilization with single and a triple doses on compost with or without PRP SOL increased the urease activity on average by 30.4 between soil sampling times I and IV. The largest increase in dehydrogenase activity was observed between soil sampling times I and III and in control objects. The applied fertilization with a triple dose of compost with or without PRP SOL increased the dehydrogenase activity on average by 18.65% between soil sampling times I and III.

The applied organic fertilization together with active substance PRP SOL stimulated the enzymatic activity of urease and dehydrogenase in all cases.

Keywords: compost, PRP SOL, soil enzymatic activity, urease, dehydrogenase

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Table 1. Some indicators of soil fertility in the Ap (0-25 cm) top layer before establishment of the experiment.

pH_{KCl}	$C_{org.}$	Total content macroelements in $g \cdot kg^{-1}$ DM soil						C:N	Content of available forms in $mg \cdot kg^{-1}$ DM soil			
		N	P	K	Ca	Mg	S		P	K	Mg	$S\text{-}SO_4$
6.65	7.55	0.64	1.10	2.41	2.18	0.60	0.12	11.8	78.2	113.2	38.6	9.26

Table 2. The physical and chemical characteristics of compost used in the field experiment.

pH_{H_2O}	Total content in $g \cdot kg^{-1}$ DM								Total content in $mg \cdot kg^{-1}$ DM				
	d.m	N	C	P	K	Ca	Mg	S	Cd	Cu	Ni	Pb	Zn
7.15	290	28.6	246	12.0	6.70	4.80	2.22	3.60	2.50	74.3	3.55	45.3	263

d.m – dry mass

Introduction

Rise in prices of mineral fertilizers and reduced production of natural fertilizers due to decreased livestock production require cheaper and environmentally safe sources of nutrients for crop production. Several studies [1-3] indicate that municipal sewage sludge contains nutrients for plants and organic matter, and attention also has been paid to the possibility of using municipal sewage sludge for production of organic fertilizers.

It has been reported [17-19] that organic and natural fertilization plays an important role because it determines soil carbon content and is generally positively correlated with the activity of enzymes [20].

Previous studies showed that active substance PRP SOL improved the soil's physical properties, increased soil enzymatic activity, and induced the conversion of sparingly soluble P, K, and Mg forms into more plant-available ones [21].

The enzymatic activity of soil can be used as a sensitive indicator in the evaluation of soil fertility and yield potential. Soil enzymatic activity depends on optimal soil moisture and aeration [4], organic carbon and nitrogen contents [5], the presence of heavy metals [6], fertilization [7-9], and to large extent on agrotechnical measures [10, 11]. Among soil enzyme activities, urease, dehdrogenase, and phosphatise activities also depend on soil type [12], physicochemical properties [13], plant species being grown [14], and their development stage [15]. Enzyme activity is particularly high in the rhizosphere, where the release of root exudates sustain a higher microbial biomass and activity than in the bulk soil [16].

The objective of this study was to determine the effect of organic fertilization with compost produced from sewage sludge with or without the addition of active substance PRP SOL on the soil urease, phosphataise, and dehydrogenase activities at different times of soil sampling during the cultivation of winter wheat (2008) and spring rapeseed (2009).

Material and Research Methods

The study was conducted in 2008 and 2009, at the Agricultural Experimental Station in Lipnik near Stargard Szczeciński, belonging to the Western Pomeranian University of Technology in Szczecin. The analyzed soil had a silty loam texture (12% clay), and included in the soil quality class IVa and good rye complex (5). Soil samples for analysis were collected from the arable layer (0-25 cm). Main soil properties are reported in Table 1. Municipal sewage sludge compost used in this study was produced by the GWDA method (GWDA Water Supply and Wastewater Disposal Services Co. Ltd, Piła, Poland) by the Municipal Sewage Treatment Plant in Stargard Szczeciński. It had a neutral pH value and its main chemical properties and heavy metal concentrations are reported in Table 2. Contents of the measured heavy metals did not exceed the Polish threshold values for its use in agriculture [22].

The experimental design included a control, with standard mineral fertilization, and three levels of organic fertilization equivalent to 100, 200, and 300 kg $N \cdot ha^{-1}$. The experiment was conducted in two series with or without the addition of active substance PRP SOL (PRP Technologies, France). The increasing doses of compost for respective fertilization soils were introduced into soil in autumn 2007. Test crops were winter wheat in 2008 and spring rapeseed in 2009.

In 2008 and 2009 the experimental area was fertilized with PRP SOL at a 150 $kg \cdot ha^{-1}$ dose and multicomponent fertilizer Polifoską 6 at a 200 $kg \cdot ha^{-1}$ dose. Due to a low content of N in Polifoska 6 (6% N), nitrogen top-dressing for winter wheat and spring rapeseed was applied in the form of urea (46% N) at a dose of 100 kg $N \cdot ha^{-1}$. The total dose of N for winter wheat (2008) and spring rapeseed (2009) was divided into two equal parts, applying them in two time periods (spring): winter wheat – 50% in the beginning of vegetation period and 50% at shooting stage, spring rapeseed – 50% before sowing and 50% before inter-row closing.

Soil samples for chemical analyses were collected from the arable layer (0-25 cm) under winter wheat four times: April, May, and July (soil sampling times I to III), and after its harvest at the end of August 2008 (soil sampling time IV). In spring 2009, spring rapeseed was sown in the same field. Soil samples for analysis were collected in the same soil sampling times I to III (i.e. April, May, and

Table 3. Effect of organic fertilization with and without the addition of active substance PRP SOL on organic carbon, total nitrogen, and phosphorus contents in soil samples collected in 2008 and 2009 following the harvest of test plants.

Fertilization soils	Study years	pH_{KCl}	Corg	N	P
			content in g·kg^{-1} d.m.		
Control	2008	6.10	7.17	0.65	1.18
	2009	6.25	7.25	0.66	1.15
Control with PRP SOL	2008	6.24	7.32	0.67	1.21
	2009	6.36	7.40	0.68	1.20
1st dose of compost without PRP SOL	2008	6.67	8.72	0.75	1.25
	2009	6.70	8.80	0.83	1.34
1st dose of compost with PRP SOL	2008	6.74	8.82	0.79	1.31
	2009	6.86	8.90	0.85	1.38
2nd dose of compost without PRP SOL	2008	6.95	8.78	0.81	1.32
	2009	7.10	8.86	0.84	1.34
2nd dose of compost with PRP SOL	2008	7.03	9.15	0.83	1.36
	2009	7.15	9.30	0.88	1.41
3rd dose of compost without PRP SOL	2008	7.08	9.04	0.85	1.37
	2009	7.20	9.10	0.87	1.39
3rd dose of compost with PRP SOL	2008	7.12	9.24	0.81	1.39
	2009	7.29	9.35	0.89	1.44
$LSD_{0.05}$:					
A – compost doses		0.21	0.15	0.08	0.09
B – PRP SOL fertilization		n.s.	0.08	n.s.	n.s.
A×B		n.s.	0.21	n.s.	n.s.

n.s. – non significant

July), while soil sampling time IV following the rapeseed harvest fell at the beginning of August 2009. Soil total organic C and total N contents were measured by sulphochromic oxidation (ISO 14235) and dry combustion (ISO 13878), soil pH was determined by potentiometry in 1M KCl (PN-ISO 10390), and total P content by the colorimetric method after previous wet mineralization of soil in a mixture of perchloric and nitric acids (1:1). Dehydrogenase activity was determined by the colorimetric method on a Lambda 150 spectrophotometer (Perkin Elmer, USA) at 485 mm wavelength after 24-hour incubation at 30ºC with TTC solution according to the method of Thalman [23]. Urease activity was determined according to the method of Zantua and Bremner [10], based on the spectrophotometric measurement of released ammonia after a 2-hour incubation of soil samples with urea 2.5% substrate at 37ºC. The phosphatase activity was determined according to Tabatabai [24].

Statistical analysis of results was performed using Statistica 8.0 computer software, while differences between means were evaluated by Tukey's at the significance level p=0.05.

Results and Discussion

Soil pH_{KCl} value, organic carbon, nitrogen, and phosphorus contents are compared in Table 3. The soil samples collected in 2008 were characterized by sub-acidic to neutral pH_{KCl} values (6.10-7.12), while those collected in 2009 by sub-acidic to sub-alkaline pH values (6.25-7.29) in the treatment with a triple dose of compost and a PRP SOL addition. Fertilization with a triple dose of compost with a PRP SOL increased the pH_{KCl} value in soil in 2008 by 1.02 units, while in 2009 by 1.04 units when compared to control soil (Table 3).

The organic carbon OC content was within the range of 7.17 to 9.35 g·kg^{-1}, while that of N 0.65 to 0.89 g·kg^{-1} and P 1.15 to 1.44 g·kg^{-1} d.m. Higher organic carbon, nitrogen, and phosphorus contents were found in the soils fertilized with double and triple doses of municipal sewage sludge compost with a PRP SOL addition.

Both in 2008 and 2009, the lowest content of three examined macroelements was determined only in the soil samples collected from control plots with or without a PRP SOL addition.

Table 4. Urease activity according to organic fertilization with and without the addition of active substance PRP SOL and soil sampling times; data are given in mg $N\text{-}NH_4 \cdot kg^{-1} \cdot h^{-1}$

Fertilization variants	Sampling times	without PRP SOL	with PRP SOL	Mean	without PRP SOL	with PRP SOL	Mean
		2008 – winter wheat			2009 – spring rapeseed		
Control	I	14.7	15.0	14.8	17.3	17.7	17.5
	II	14.5	15.2	14.8	17.4	17.8	17.6
	III	14.8	15.1	14.9	17.4	17.6	17.6
	IV	14.9	15.3	15.1	17.5	17.9	17.7
	Mean	14.7	15.1	14.9	17.4	17.7	17.6
1st dose of compost	I	15.0	15.2	15.1	18.9	19.0	19.0
	II	15.4	15.4	15.4	19.2	19.4	19.3
	III	15.1	15.3	15.2	19.1	19.2	19.1
	IV	15.2	15.6	15.3	19.6	19.8	19.7
	Mean	15.2	15.4	15.3	19.2	19.3	19.2
2nd dose of compost	I	15.2	15.5	15.4	19.1	19.2	19.1
	II	15.6	15.8	15.7	19.5	19.6	19.5
	III	15.4	15.6	15.5	19.3	19.3	19.3
	IV	15.9	16.2	16.1	19.7	19.9	19.8
	Mean	15.5	15.8	15.6	19.4	19.5	19.4
3rd dose of compost	I	15.7	15.9	15.8	19.3	19.6	19.4
	II	15.9	16.1	16.0	19.7	19.9	19.8
	III	15.7	16.3	16.0	19.5	20.2	19.8
	IV	16.1	16.4	16.2	20.1	21.1	20.6
	Mean	15.8	16.2	16.0	19.6	20.2	19.9
Control	I	14.7	15.0	14.8	17.3	17.7	17.5
	II	14.5	15.2	14.8	17.4	17.8	17.6
	III	14.8	15.1	14.9	17.4	17.6	17.6
	IV	14.9	15.3	15.1	17.5	17.9	17.7
	Mean	14.7	15.1	14.9	17.4	17.7	17.6
$LSD_{0.05}$							
A – compost doses		0.166			0.267		
B –PRP SOL fertilization		0.088			0.141		
A×B		n.s.			n.s.		

n.s. – non significant

The applied organic fertilization with compost with or without the addition of PRP SOL significantly increased the OC content in all fertilization soils. Nitrogen and phosphorus contents in soil after plant harvest increased significantly in the soils being fertilized with increasing compost doses when compared to control soils. The introduction of active substance PRP SOL into soil did not contribute to a significant increase in the content of both chemical elements in the soil under analysis (Table 3).

Urease and dehydrogenase activities at different sampling times are reported in Tables 4 and 5. The increasing doses of compost with or without PRP SOL and soil sampling times in the two study years significantly affected urease and dehydrogenase activities. The variability of enzymatic activities in soil during the crop vegetation period, mainly due to changing temperature and precipitation, has been previously reported [25], and agrees with previous published data that soil enzymes are more active at the end

Table 5. Dehydrogenase activity according to organic fertilization with and without active substance PRP SOL and soil sampling times; data are given in $cm \cdot H_2 \cdot kg^{-1} \cdot d^{-1}$.

Fertilization variants	Sampling times	without PRP SOL	with PRP SOL	Mean	without PRP SOL	with PRP SOL	Mean
		2008 – winter wheat			2009 – spring rapeseed		
Control	I	11.4	11.7	11.5	12.2	13.8	13.0
	II	11.5	11.8	11.6	12.3	13.9	13.1
	III	11.6	11.9	11.7	12.4	13.9	13.1
	IV	11.7	12.0	11.8	12.5	14.0	13.2
	Mean	11.6	11.8	11.7	12.4	13.9	13.1
1st dose of compost	I	12.2	12.9	12.5	13.2	14.1	13.6
	II	12.7	13.2	12.9	13.5	14.6	14.0
	III	13.2	13.7	13.4	13.9	14.9	14.4
	IV	12.9	13.5	13.2	13.7	14.9	14.3
	Mean	12.7	13.3	13.0	13.6	14.6	14.1
2nd dose of compost	I	12.8	13.0	12.9	13.8	14.2	14.0
	II	13.1	13.5	13.3	14.1	14.6	14.3
	III	13.7	14.1	13.9	14.5	15.0	14.7
	IV	13.4	13.8	13.6	14.1	14.9	14.5
	Mean	13.2	13.6	13.4	14.1	14.7	14.4
3rd dose of compost	I	13.2	13.7	13.4	14.3	15.9	15.1
	II	13.6	13.9	13.7	14.7	16.3	15.5
	III	14.1	14.3	14.2	15.1	16.7	15.9
	IV	13.8	14.1	13.9	14.9	16.6	15.7
	Mean	13.7	14.0	13.8	14.7	16.4	14.0
$LSD_{0.05}$							
A – compost doses		0.203			0.185		
B –PRP SOL fertilization		0.107			0.097		
A×B		n.s.			0.261		

n.s. – non significant

of spring and the beginning of summer and autumn [26]. In both study years, the highest activity of urease was found in the soil samples collected in July.

The obtained study results are likely related to site temperature, which was almost the same in July in 2008 and 2009 and amounted to 19.4 and 19.1ºC, whereas total precipitation in mm was slightly higher in 2009 (53.7 mm) when compared to 2008 (35.2 mm).

The application of increasing doses of municipal sewage sludge compost with the addition of PRP SOL and the soil sampling times were both the parameters significantly influencing the soil urease and dehydrogenase activities (Tables 4 and 5).

The urease activity ranged from 14.5 to 21.1 mg $N\text{-}NH_4 \cdot kg^{-1} \cdot h^{-1}$. The soil samples collected in 2009 under spring rapeseed showed higher urease activity when compared to that determined in the samples under winter wheat. Urease and dehydrogenase activities in the soil samples collected under spring rapeseed and winter wheat in 2009 were higher than those in 2008 (Tables 4 and 5). This phenomenon could be induced by different nutrient uptake (particularly N) by the plant roots as well as by different times of the urea top-dressing application. This hypothesis agrees with previous studies by Yang et al. [27], who state that urease activity may be inhibited by the application of fertilizers containing ammonium N.

Urease activity in the soil under winter wheat increased respectively by 4.51% and 4.60% between soil sampling times I and IV (April-August) in the soils receiving a double dose of compost, independently of PRP SOL addition.

Organic fertilization with compost significantly increased the OC, total N, and P contents in all soils, regardless of the addition of PRP SOL.

A similar trend was observed in the soil fertilized with a triple dose of compost with or without the PRP SOL addition. An increase of urease activity was 2.54% and 3.14% in soils with double and triple compost doses, respectively, compared to soils receiving a double dose of compost.

The highest urease activity in the soil samples collected under spring rapeseed was characteristic of the object fertilized with a tripled dose of compost with PRP SOL addition between soil sampling times I and IV (April-August).

In this fertilization object, the activity of urease increased by 7.65%. A slightly smaller increase, by 4.14%, was found in the object fertilized with a triple dose of compost only (Table 4).

The largest increase in the activity of that enzyme was found between soil sampling times IV and I. The highest urease activity was observed in soils receiving a triple dose of compost, independently on PRP SOL addition both for winter wheat and spring rapeseed. A difference in soil urease activity between soils receiving fertilizers and those receiving sludge-derived compost was significant for 2008. Differences in soil enzymatic activity between fertilization soils with single compost dose in 2009 were not significant.

When comparing the urease activity in soil samples, a significantly larger effect of organic fertilization with or without PRP SOL addition under spring rapeseed was found when compared to the soil material collected under winter wheat.

The dehydrogenase activity varied within the range of 11.4-16.7 $cm \cdot H_2 \cdot kg^{-1} \cdot d^{-1}$ and the soils collected in 2009 under spring rapeseed showed higher dehydrogenase activity when compared to soils under winter wheat. Higher dehydrogenase activity was found in soils under spring rapeseed as compared to soils under winter wheat (Table 5).

Dehydrogenase activity in the soil samples collected under winter wheat increased by 8.20%, 7.03%, and 6.82% between soil sampling times I and IV in the soils treated with single, double, and triple doses of compost, respectively. The highest dehydrogenase activity in the soil samples collected under spring rapeseed was characteristic of the soil fertilized with single and double doses of compost with PRP SOL and with a triple dose of the compost itself between soil sampling times I and III (April-July). In these fertilized soils and sampling times, dehydrogenase activity increased by 5.67%, 5.63%, and 5.59%, respectively (Table 5).

In the control soil, the largest increase in dehydrogenase activity was found between soil sampling times III and I (Table 5). On average, the application of a triple dose of compost with or without a PRP SOL addition increased the dehydrogenase activity by 18.65% between soil sampling times III and I, whereas an average increase of dehydrogenase activity in the control soil with PRP SOL was 16.9% between 2008 and 2009.

Urease and dehydrogenase activities in the soil samples collected under both test plants were significantly higher in the fertilization soils where compost and the addition of active substance PRP SOL had been applied when compared to the control object with or without a PRP SOL addition (Table 4 and 5).

Depending on the crop and the sampling time, urease and dehydrogenase activities were higher by 2.04% to 21.3% and 5.17% to 34.7%, respectively, when compared to the control object (Tables 4 and 5). With the presented experimental set-up it was not possible to discriminate the effect of microorganisms introduced into soil with the sludge-derived compost [28] on the whole soil enzyme activity.

Conclusions

1. Fertilization with a triple dose of compost with a PRP SOL addition significantly increased the soil pH value as compared to control soils.
2. Fertilization with increasing doses of compost with PRP SOL under winter wheat and spring rapeseed increased the urease activity between soil sampling times I and IV, by 4.96% and 11.97%, respectively, when compared to the control soil.
3. The largest increase in dehydrogenase activity in soil material was observed between soil sampling times I and III as affected by organic fertilization with a PRP®SOL addition under winter wheat and spring rapeseed, by 14.5% and 11.0%, respectively, when compared to the control soil.
4. Depending on the applied fertilization, the crop, and sampling time, urease and dehydrogenase activities were higher by 2.04% to 21.3% and 5.17% to 34.7%, respectively, when compared to the control soil.
5. The application of organic fertilization with active substance PRP SOL stimulated the enzymatic activity of urease and dehydrogenase in all fertilization objects.

References

1. BARAN S. Resources and organic waste management in Poland. Selected aspects of the management of organic waste and biomass production of energy willow. Wydaw. URzeszow., Rzeszów, pp. 17-40, **2005**.
2. KRZYWY E., ZUBER B., KAŹMIERCZAK A. Effect of fertilization on the substance PRP soil fertility and productivity. Riazanskij Gosudorstwiennyj Medicinskij Uniwersytet, pp. 108-114, **2005**.
3. WOŁOSZYK C. Agrochemical evaluation of fertilization with composts of municipal sewage sludge and industrial waste. Habilitation dissertation 217. Wyd. AR w Szczecinie, 120, **2003**.
4. CIEŚLA W., KOPER J. Effect of multiple mineral-organic fertilization on the formation of the level of available phosphorus and organic and enzymatic activity of soil. Rocz. Gleboz. **41**, 73, **1990**.
5. DICK R.P. A review: long-term effect of agricultural system on soil biochemical and microbil parameters. Agr. Ecosyst. Environ. **40**, (1-4), 25, **1992**.
6. MENCH M., RENELLA G., GELSOMINO A., LANDI L., NANNIPIERI P. Biochemical parameters and bacterial species richness in soils contaminated by sludge – borne metals and remediated with inorganic soil amendments. Environ. Pollut. **144**, 24, **2006**.

7. KUCHARSKI J., WŁDOWSKA E. Biochemical effects of long-term fertilization with slurry. Zesz. Prol. Post. Nauk Rol. **476**, 196, **2001**.
8. WYSZKOWSKA J., KUCHARSKI J. Fertilization with straw and sawdust as a factor in the leveling effects of soil contamination with cadmium to microorganisms Zesz. Prol. Post. Nauk Rol. **506**, 557, **2005**.
9. ZANTUA M.I., BREMNER J.M. Comparison of methods of assaying urease activity in soils. Soil Biol. Biochem. **7**, 291, **1975**.
10. BIELIŃSKA E.J., ŻUKOWSKA G. Protease and urease activity in light soil fertilized with sewage sludge. Acta Agrophysica **70**, 41, **2002**.
11. BIELIŃSKA E.J., BARAN S., DOMŻAŁ H. The use of indicators to assess the effects of enzymatic annual agrotechnical improve the properties of light soil. Folia Univ. Agric. Stetin. Ser. Agriculturae **211**, (84), 35, **2000**.
12. RENELLA G., LANDI L., VALORI F., NANNIPIERI P. Microbial and hdrolase activity after relase of low molecular weght organic compounds by a model root surface in a clayey and a sand soil. Appl. Soil Ecol. **36**, 124, **2007**.
13. NORTCLIFF S. Standarisation of soil quality attributes. Agric. Ekosys. Environ. **88**, 161, **2002**.
14. TRASAR-CEPEDA C., LEIROS M.C., GIL-SOTRES F. Hydrolytic enzyme activities in agricultural and forest soils. Some implications for their use a indicators of soil quality. Soil Biol. Biochem. **40**, 2146, **2008**.
15. YANG L., LI T., LI F., LEMCOFF J.H., COHEN S. Fertilization regulates soil enzymatic activity and ferility dynamics in cucumber field. Sci. Hortic. **116**, 21, **2008**.
16. RENELLA G., CHAUDRI A.M., FALLOON C.M., LANDI L., NANNIPIERI P., BROOKES P.C. Effects of Cd, Zn, or both on soil microbial biomass and activity in a clay loam soil. Biol. Fert. Soils **43**, 751, **2007**.
17. DAR G.H. Effects of cadmium and sewage- sludge on soil microbial biomass and enzyme activities. Biores. Technol. **56**, 141, **1996**.
18. SAHA S., PRAKASH V., KUNDU S., KUMAR N., MINA B.L. Soil enzymatic activity as affected by lond term application of farm yard manure and mineral fertilizer under a rainfed soyebeanwheat system in N-W Himalaya. Europ. J. Soil Biol. **44**, 309, **2008**.
19. ZHAO Y., WANG P., LI J., CHEN Y., YING X., LIU S. 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. **2009**.
20. VENKATESAN S., SENTHURPANDIAN V.K. Comparision od enzyme activity with depth under tee plantations and forested sites in south India. Geoderma **137**, 212, **2006**.
21. KRZYWY-GAWROŃSKA E. The study on the influence of the compost from the municipal sewage sludge and used against of them of active substance of PRP Sol on the fertility and productivity of the soil. Wyd. ZUT w Szczecinie pp. 96, **2009**.
22. Official Journal of Laws No. 119, item 765, **2008**.
23. THALMANN Methodology for determination of dehydrogenase activity in particular by means of ground. Triphenyltetrazolium chloride (TTC). Agricultural Machines. **21**, 249, **1968** [In German].
24. TABATABAI M.A., BREMNER J.M. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biol. Biochem. **1**, 301, **1969**.
25. GIANFREDA L., RUGGIERO P. Enzyme activities in soil. W. P. Nannipieri, K. Smalla (Ed.): Soil biology. Nucleic acids and proteins in soil. Springer-Verlang **8**, 257, **2006**.
26. JANUSZEK K. Seasonal changes of enzyme activity in mor, moder and mull humus of selected forest soils in the Western Beskid Mountains. Fol. For. Pol. **3**, 59, **1993**.
27. KIELISZEWSKA–ROKICKA B. Soil enzymes and their importance in studies of soil microbial activity. W. H. Dahm. A. Pokojska-Brawl (Ed.). Microorganisms of the soil environment. Toruń: pp. 37-47, **2001**.
28. GILEWSKA M. Biological Reclamation of land ash landfills ZEPAK SA Materials X Jubilee International Conf. “Ashes of energy” pp. 331-342, **2006**.