

## Effect of Fertilization with Sludge-Ash Granulates on the Activity of Selected Soil Enzymes

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### ABSTRACT

In the two-factorial vegetation experiment, the activity of dehydrogenase, phosphatase and urease was examined under the influence of the applied sludge-ash granulates produced from waste (sewage sludge, ash, sawdust). The research scheme included a control object, four types of fertilizer granulates, three doses and four dates of enzymatic activity indicators determination. The experiment was established in four replications. The size of granulate doses was determined on the basis of their nitrogen content. Dose I, II and III were: 0.24 g, 0.48 g and 0.72 g N-pot, respectively. The test plant was rapeseed of *Larissa* cultivar. The sludge-ash granulates were applied to the pots filled with the soil, in accordance with the experimental scheme. The soil samples for chemical analyses were taken from the top layer (0–20 cm) of spring rape cultivation four times: May 16, June 2, July 2 – dates from I to III, respectively, and August 2 after harvest – date IV. In average soil samples, the enzymatic activity of dehydrogenase, urease and phosphatase was determined. The research revealed that the highest increase in enzymatic dehydrogenase activity was obtained after applying granulate B, while phosphatases and urease after the application of granulate D. The smallest increase in dehydrogenase and phosphatase activity was obtained after the application of granulate C. The dose size of the applied granulates and date of uptake had a significant impact on the growth activity of the enzymes studied. The activity of dehydrogenase, phosphatases and urease, depending on the type of applied granulate, dose and date of soil sampling, was on average 7.28%, 30.5% and 7.94% higher, respectively, compared to the control. The correlation coefficient between dehydrogenase activity and urease and phosphatase was positively associated and amounted to  $r = 0.569$  and  $r = 0.553$ , respectively. The applied fertilization with sludge-ash granulates A, B, C and D stimulated the increase in dehydrogenase, urease, and phosphatase activities in all fertilizer objects.

**Keywords:** soil, granulates sludge-ash, enzymatic activity phosphatase, urease, dehydrogenase.

### INTRODUCTION

The applicable legislation related to environmental protection imposes an obligation on producers or importers to develop or dispose of the generated waste. Waste disposal is associated with financial costs. Therefore, producers of sewage sludge, knowing their fertilizing properties (they contain organic substances and some nutrients for plants), provide them for secondary use in order to fertilize the soils [Antonkiewicz 2010]. In addition, sewage sludge applied to light soils in optimal doses contributes to increasing its enzymatic activity [Antonkiewicz

et al., 2018, He et al. 2009, Krzywy et al. 2012, Kulikowski et al. 2019]. Ashes from the coal combustion constitute another waste produced in significant quantities that is stored and managed in various ways. According to Łabętowicz et al. [2019] they have positive effect on soil properties, among others: they increase the content of some macroelements and micronutrients, as well as CaO and MgO, moisture content, and contribute to de-acidification. Bielińska et al. [2009] indicate attention should be paid to the low permeability of coal ash and their negative impact on the activity of some enzymes in soils. Despite these drawbacks,

results of many studies indicate that ashes used in optimal doses give better results than typical calcium fertilizers [Antonkiewicz 2009]. They are also an abundant source of some nutrients for plants, and create optimal conditions for the development of many soil microorganisms, which has a positive influence. Therefore, attention was paid to the possibility of using the sewage sludge, ashes and sawdust for the production of sludge-ash granulates, which may lead to the increased fertility of soils and soil enzymes activity [Możdżer et al. 2020, Łabętowicz et al. 2019].

Level of enzymatic activity is a fairly sensitive indicator of the soil fertility assessment [Lemanowicz and Koper 2012]. The activity of soil enzymes depends on the optimal humidity, the access of air to soils, type of soil, concentration of heavy metals, pH values, nitrogen and carbon content, cultivated plant species and its development stages [Bielińska et al. 2013, Trasar-Cepeda et al., 2008, Renella et al. 2007, Wyszowska et al. 2009, Telesiński et al. 2012]. It is an indicator of ecological changes occurring in the soil environment. Changes in the soil environment depend to a greater extent on the activity of individual enzymes calculated per unit of organic carbon than the activity expressed in substrate units per unit soil mass. Organic fertilization is of great importance, because it determines the carbon content in the soil and, in the majority of cases, it is positively correlated with the activity of enzymes [Kalembasa et al. 2014].

The research aimed to determine the impact of four sludge-ash granulates applied in three doses and collected in four dates, on the activity of soil enzymes: dehydrogenase, phosphatases and urease, during the cultivation of spring rape of *Larissa cv.*

## MATERIAL AND METHODS

The sludge-ash granulates used for the research were produced from industrial sewage sludge in sewage treatment plants Zakłady Chemiczne Police Grupa Azoty in Police, ash from brown coal combustion in Adamów S.A. power plant, and sawdust from deciduous and coniferous trees. On the basis of the chemical properties of sewage sludge, ash from lignite and sawdust, material compositions of four sludge-ash granulates were developed. Table 1 shows the chemical composition of the produced ash-sediment granulates.

In order to obtain the optimal N:P:K ratio, ammonium phosphate and potassium salt were added to the granulates. The vegetative-pot experiment with the participation of produced granulates was carried out in the Vegetation Hall belonging to ZUT in Szczecin. The soil from the Ap level of the production field of the Agricultural Experimental Station in Lipnik near Stargard was used for the research. This soil is classified as incomplete brown with granulometric composition of strong loamy sand with a content of 12% of the alluvial parts. In terms of agricultural suitability, the soil belongs to the good rye complex of bonitation class IVb. It was characterized by slightly acidic pH, narrow C:N ratio and low organic carbon content. The content of bioavailable forms for phosphorus, potassium and magnesium plants was medium (Table 2).

The research scheme included control object, four types of sludge-ash granulates, three doses and four dates of enzyme activity indicators determination. The experiment was established in four replications. Dose I, II and III amounted to: 0.24 g N·pot (80 kg N·ha<sup>-1</sup>), 0.48 g N·pot (160 kg N·ha<sup>-1</sup>) and 0.72 g N·pot (240 kg N·ha<sup>-1</sup>), respectively. The test plant was rapeseed of *Larissa* cultivar. The size of granulate doses was determined on the basis of their nitrogen content. In addition to granulates, nitrogen supplementation was applied in the form of an aqueous urea solution.

**Table 1.** Material composition of fertilizer blends expressed in % dry matter. Origin: Możdżer and Jałoszyński [2019], Możdżer et al. [2020]

Type of granulate	Industrial sewage sludge	Brown coal ash	Sawdust	Ammonia phosphate	Potassium salt
In %					
A	30	30	10	15	15
B	40	20	10	15	15
C	20	40	10	15	15
D	50	20	10	10	10

**Table 2.** The content of the macroelements in the soil used in the experiment. Origin: Możdżer and Jałoszyński [2019], Możdżer et al. [2020].

pH <sub>KCl</sub>	Total content							C:N	Content of assimilable forms		
	Total content in g·kg <sup>-1</sup> d.m.								In mg·kg <sup>-1</sup> d.m.		
	C <sub>org.</sub>	N	P	K	Ca	Mg	S		P	K	Mg
6.0	9.63	0.86	1.50	2.57	2.11	0.56	1.25	11.2	60.9	121	42.5

Rape was fertilized five weeks after emergence. The dose amount corresponded to 0.276 g N·pot<sup>-1</sup>. The sludge-ash granulates were applied to the pots filled with soil in accordance with the experimental pattern. Then, 20 rape seeds were sown. After reaching 10 cm test plant height, selection was made, leaving 5 rapeseed plants in each pots.

In order to investigate the impact of individual sludge-ash granulates on soil enzymatic activity, samples were collected at specific intervals during the development of spring rape. They were taken from the top layer (0–20 cm) of rapeseed plantation four times: May 16, June 2, July 2 – dates marked from I to III, respectively, and August 2 after harvesting the test plant – date IV. Dehydrogenase activity was determined colorimetrically on a Lambda 150 spectrophotometer at a wavelength of 485 nm after 24-hour incubation at 30°C with a TTC solution, according to Thalmana [1969]. Urease activity was determined according to the method based on spectrophotometric measurement of released ammonia after a 2-hour incubation of the soil sample with urea at the concentration of 2.5% as a substrate at 37°C-Zantua and Bremner [1975]; phosphatase according to the Tabatabai [1994] method, organic carbon (ISO 14235), total nitrogen (ISO 13878), pH - potentiometrically in 1 mol KCl solution. The statistical analysis of results was performed using the Statistica 13 software, and differences between the average values were assessed by the Tukey test at the significance of  $p = 0.05$ . The material composition of granules and the chemical properties of the soil were published before the experiment was set up in Możdżer and Jałoszyński [2019], Możdżer et al. [2020].

## RESULTS AND DISCUSSION

The changes of enzymatic processes occurring in the soil during the growing season of plants are characteristic, because they depend on the prevailing atmospheric conditions. It should be noted that enzymes are more active at the end of spring as well as the beginning of summer and

autumn [Yang et al., 2008, Krzywy-Gawońska 2012, Kalembsa et al. 2014].

Dehydrogenase, phosphatase and urease activity on various dates of soil sampling are summarized in Tables 3 and 4. The applied sludge-ash granulates A, B, C and D and increasing doses significantly influenced the increase of activity of tested enzymes. The activity of soil dehydrogenase under the influence of applied sludge-ash granulates A, B, C and D ranged from 9.15 to 10.2 cm<sup>3</sup>·H<sub>2</sub>·kg<sup>-1</sup>·d<sup>-1</sup>. The soil collected from rape plantation on date IV showed greater dehydrogenase activity compared to date I. Dehydrogenase activity higher by 2.0% was exhibited by the soil on the objects with applied granulate B (40% sewage sludge, 40% ash, 10% sawdust and 15% ammonium phosphate and KCl) in comparison with its activity obtained under the influence of applied granulate C (20% sewage sludge, 40% ash, 10% sawdust and 15% ammonium phosphate and KCl). It can be concluded that higher proportion of sewage sludge and smaller of ash (granulate B) affected the growth of tested enzyme. When analyzing the date of soil sampling, the highest dehydrogenase activity was observed in date IV (August) compared to the first term (May) under the influence of all applied granulates. The soil dehydrogenase activity under the influence of A, B, C and D granulates introduced between harvest dates I and IV (May, August) increased by 3.51%, 4.02%, 2.57% and 2.76%, respectively. The highest dehydrogenase activity was recorded under the influence of the applied granulates A and C, between the applied dose 1 and 3, by 6.31% and 7.64%, respectively, and the lowest by 3.05% under the influence of granulate D (50% sewage sludge, 20% ash, 10% sawdust, ammonium phosphate and KCl). The phosphatase activity in the soil under the influence of applied sludge-ash granulates A, B, C and D ranged from 15.62 to 16.98 mmol PNP·kg<sup>-1</sup>·h<sup>-1</sup> (Table 3).

Single and triple dose of granulate D used, doubled granulate A and tripled granulate C caused the highest increase in phosphatase

**Table 3.** The impact of sludge-ash granulates on the activity of dehydrogenase ( $\text{cm}^3\cdot\text{H}_2\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$ ) and phosphatase ( $\text{mmol PNP}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ ) in the soil

Type granulates*	Dose **																		
	1					2					3					Mean			
	Sampling times																		
	I	II	III	IV	mean	I	II	III	IV	mean	I	II	III	IV	mean	I	II	III	IV
Dehydrogenase in $\text{cm}^3\cdot\text{H}_2\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$																			
A	9.15	9.25	9.48	9.55	9.35	9.30	9.41	9.52	9.59	9.45	9.80	9.89	9.96	10.1	9.94	9.41	9.51	9.70	9.74
B	9.31	9.42	9.49	9.62	9.46	9.38	9.48	9.63	9.71	9.55	9.71	9.82	9.93	10.2	9.91	9.46	9.57	9.75	9.84
C	9.05	9.10	9.21	9.27	9.16	9.24	9.29	9.38	9.47	9.35	9.73	9.82	9.90	10.0	9.86	9.34	9.40	9.54	9.58
D	9.37	9.42	9.55	9.69	9.51	9.18	9.25	9.31	9.36	9.27	9.68	9.74	9.81	9.98	9.80	9.41	9.47	9.62	9.67
Mean	9.22	9.29	9.43	9.53	9.37	9.27	9.36	9.46	9.53	9.41	9.73	9.81	9.90	10.1	9.88	9.40	9.48	9.65	9.72
Control	8.90																		
LSD <sub>0.05</sub> for: type granulates – 0.047, dose – 0.037, interaction – 0.081.																			
Phosphatase in $\text{mmol PNP}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$																			
A	15.80	15.92	15.96	16.12	15.9	16.03	16.11	16.21	16.32	16.2	16.38	16.49	16.58	16.62	16.5	16.07	16.17	16.34	16.35
B	15.91	16.05	16.12	16.21	16.1	15.97	16.03	16.12	16.22	16.1	16.31	16.41	16.52	16.58	16.4	16.06	16.16	16.31	16.33
C	15.62	15.67	15.71	15.82	15.7	15.82	15.91	15.98	16.06	15.9	16.47	16.54	16.63	16.79	16.6	15.97	16.04	16.17	16.22
D	16.71	16.78	16.89	16.98	16.8	15.93	15.98	16.06	16.19	16.0	16.54	16.36	16.71	16.89	16.6	16.39	16.37	16.62	16.68
Mean	16.01	16.1	16.2	16.3	16.1	15.9	16.0	16.1	16.2	16.0	16.4	16.5	16.6	16.7	16.5	16.10	16.2	16.36	16.40
Control	15.1																		
LSD <sub>0.05</sub> for: type granulates – 0.054, dose – 0.043, interaction – 0.094.																			

\* Explanations of the material composition of sludge-ash granulates are given as in Table 1.

\*\* The size of granulated doses and the dates of soil samples collection are given “Material and method”.

activity compared to other fertilizer objects. The type of applied granulates A, B and C had a significant effect on the increase in phosphatase activity between 1 and 3 doses, by 3.77%, 1.86% and 5.73%, respectively. On the other hand, the introduced doubled dose of granulate D caused a decrease in the activity of phosphatases compared to the single dose by 5.0%. When analyzing the date of soil samples collection, the highest phosphatase activity was found in date IV compared to dates I, II, and III. The increase in phosphatase activity in the soil under the influence of granulates A, B, C and D introduced between dates of sampling 1 and 4 was small and amounted to 1.74%, 1.68%, 1.56% and 1.76%, respectively. Bielińska and Mocek-Płóćiniak [2009] indicate that lower phosphatase activity may be caused by the introduction of calcium, which was confirmed in our own research in the case of the applied C. Urease activity ranged from 7.29 to 9.12  $\text{mg N-NH}_4\cdot\text{kg}^{-1}\cdot\text{ha}^{-1}$ . The applied single and triple dose of granulate B and doubled granulate D caused the greatest increase in the activity of the tested enzyme in comparison to other fertilizer objects (Table 4).

When analyzing the date of soil sampling, it was found that under the influence of A, B, C and D granulates, the urease activity in soil between the I and IV harvest (May, August) increased by 2.89%, 2.23%, 3.73% and 4.33%, respectively. The soil collected in date IV, under the influence

of applied granulates, showed the highest urease activity in comparison with date I and the control. The obtained results of the research are most likely related to the air temperature, which in the examined period (August) was optimal for the development of enzymes and fluctuated from 19.4 to 19.1°C. The highest urease activity in the soil was recorded under the influence of granulates C and D between doses 1 and 2 by 19.0% and 20.0%, respectively, and the decrease in activity between doses 2 and 3 by 6.13% and 6.44%, respectively. It was found that the type of applied granulates A, B, C and D had the significant effect on the increase in urease activity between 1 and 3 doses by 7.98%, 8.16%, 12.1% and 12.8%, respectively. It could have been caused by a greater uptake of free nitrogen from the air by rape, which was confirmed by the tests carried out. It should be remembered that urease activity can be inhibited by the use of nitrogen fertilizers in ammonium form [Piotrowska and Koper 2007].

The activity of dehydrogenase, phosphatases and urease, depending on the type of applied granulate, the dose and date of soil samples collection, was on average 7.28%, 7.94% and 30.5% higher than in the control.

Figure 1 shows the relationship between enzymatic activity of dehydrogenase, phosphatase and urease in soil after the use of sludge-ash granulates with varying share of sewage sludge and ash.

**Table 4.** The impact of sludge-ash granulates on the activity of urease ( $\text{mg}\cdot\text{N}\cdot\text{NH}_4\cdot\text{kg}^{-1}\cdot\text{ha}^{-1}$ ) in the soil.

Type granulates*	Dose **																		
	1					2					3					Mean			
	Sampling times																		
	I	II	III	IV	mean	I	II	III	IV	mean	I	II	III	IV	mean	I	II	III	IV
Urease in $\text{mg}\cdot\text{N}\cdot\text{NH}_4\cdot\text{kg}^{-1}\cdot\text{ha}^{-1}$																			
A	7.73	7.69	7.78	7.85	7.76	7.88	7.95	8.09	8.21	8.03	8.25	8.35	8.41	8.50	8.38	7.95	7.99	8.15	8.18
B	7.77	7.81	7.87	7.91	7.84	8.03	8.11	8.15	8.21	8.12	8.38	8.40	8.51	8.62	8.48	8.06	8.10	8.21	8.24
C	7.29	7.39	7.42	7.56	7.41	8.66	8.78	8.89	8.97	8.82	8.15	8.28	8.35	8.48	8.31	8.03	8.15	8.29	8.33
D	7.31	7.36	7.48	7.59	7.43	8.71	8.89	8.97	9.12	8.92	8.21	8.34	8.41	8.56	8.38	8.07	8.19	8.37	8.42
Mean	7.52	7.56	7.63	7.72	7.61	8.32	8.43	8.52	8.62	8.47	8.24	8.34	8.42	8.54	8.39	8.02	8.11	8.25	8.29
Control	6.25																		

LSD<sub>0.05</sub> for: type granulates – 0.045, dose – 0.035, interaction – 0.078.

\* Explanations of the material composition of sludge-ash granulates are given as in Table 1.

\*\* The size of granulated doses and the dates of soil samples collection are given “Material and method”.

While analyzing the correlation coefficients  $r = 0.707$  at  $p < 0.05$ , it was noted that the absolute phosphatase activity was positively correlated with urease activity. The correlation coefficient between dehydrogenase and urease and phosphatases activities was smaller but also positively correlated and amounted to  $r = 0.569$  and  $r = 0.553$ , respectively. Similar results were obtained by Antonkiewicz et al. [2017], Krzywy-Gawrońska et al. [2009] and Wołoszyk et al. [2006]. The abovementioned relationships indicate the effect on soil enzymatic activity of applied sludge-ash granulates A, B, C and D. It is assumed that the stabilizing effect on urease, phosphatase and dehydrogenase is affected by both sewage sludge and ash from lignite, in which calcium and magnesium carbonates are present. The correlations between the activity of soil enzymes and the content of macroelements confirm the importance of intensity of nutrient removal from the soil environment [Pogrzeba et al. 2018, Wierzbowska et al. 2016]. Growing and fertilizing soil changes its physicochemical properties and increases its enzymatic activity. Kuziemska [2012] found that organic fertilization caused a significant increase in the activity of soil enzymes. Similar results were obtained as a result of granulate D application, which contributed to the greatest extent to the increase of phosphatase and urease.

To sum up, the highest increase in enzymatic activity of dehydrogenase was obtained after the application of granulate B produced with 40% sewage sludge, 20% ash, 10% sawdust and 15% ammonium phosphate and KCl, and phosphatases and urease after using granulate D (50% sewage sludge, 20% ash, 10% sawdust, ammonium phosphate and KCl). The smallest increases in

dehydrogenase activity and phosphatases were obtained after the application of granulate C (20% sewage sludge, 40% ash, 10% sawdust and 15% ammonium phosphate and KCl). Waste in the form of sewage sludge, ash and sawdust used to produce granulates contributed to the increased activity of soil enzymes. The upward trend in enzyme activity under the influence of increasing doses of sewage sludge or composts with their participation is confirmed by many studies [Antonkiewicz et al. 2017, Krzywy-Gawrońska et al. 2009].

It was found that the granulates produced with the use of sewage sludge contain organic colloids and microorganisms that stimulate the activity of soil enzymes. Microorganisms support the use of soil macroelements by plants. Similar results were obtained by Pogrzeba et al. [2018]. Studies have shown that the dehydrogenase, phosphatase and urease activity, depending on the type of granulate applied and the size of dose, contributed – to a greater or lesser extent – to the increase in the enzymatic activity of soil. The research carried out by Antonkiewicz [2017], Bielińska et al. [2009], Wyszowska et al. [2009] confirms the increase in the enzymatic activity of the soil as a result of the application of increasing doses of sludge and ash granules. The conducted experiment showed an increase in the activity of studied enzymes, which confirms the effectiveness of using the sewage sludge as a fertilizer in the cultivation of plants [Krzywy-Gawrońska 2012, Kołodziej et al. 2015, Kołodziej et al. 2016]. The research conducted by Synamowicz et al. [2018] confirms that mineral and organic fertilization increases the soil enzymatic activity, which was proven by the conducted research.

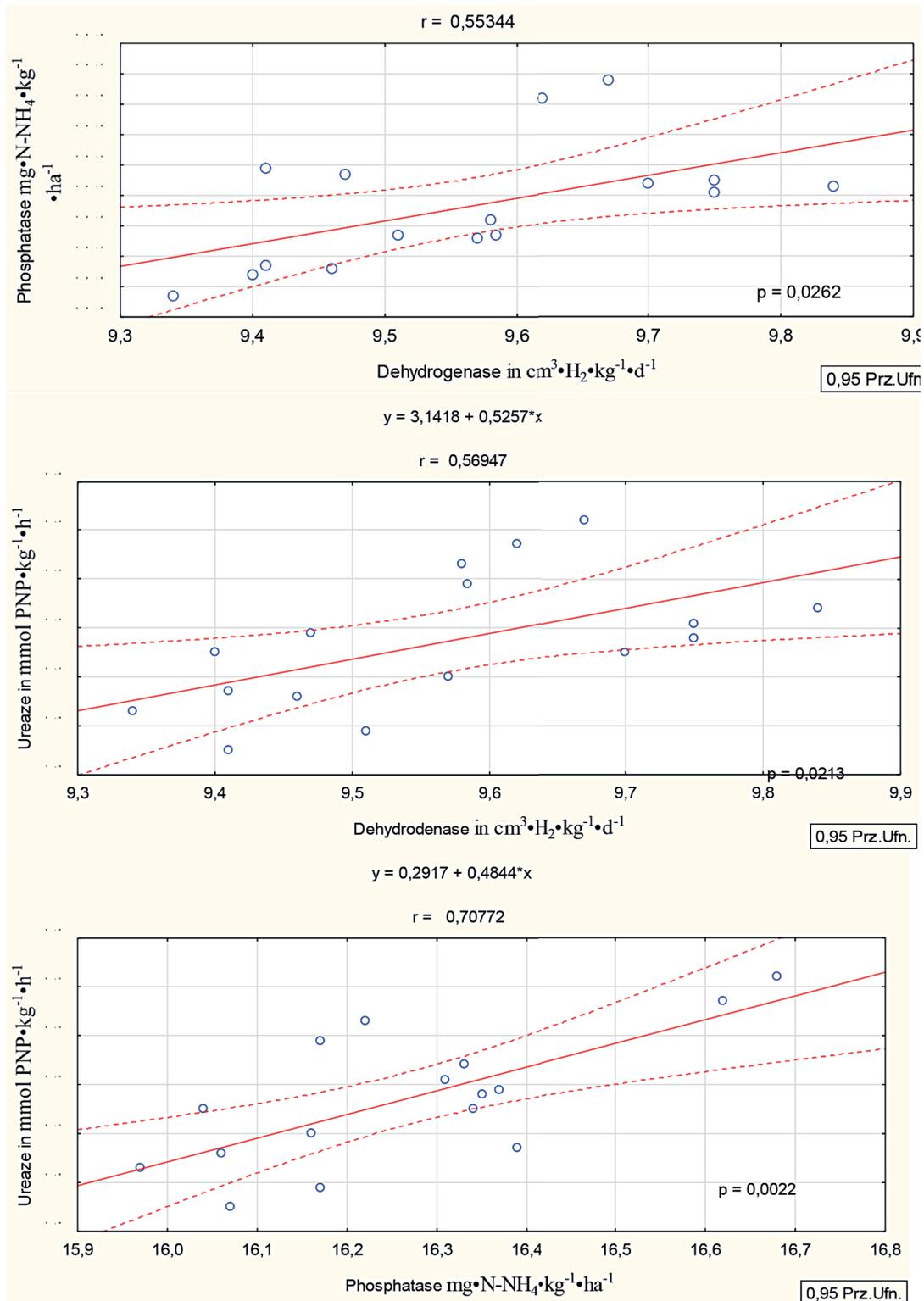


Figure 1. Relations between enzymatic activity of dehydrogenase, phosphatases and urease under the influence of applied sludge-ash granulates

## CONCLUSIONS

Soil with applied sludge-ash granulates A, B, C and D was characterized by higher activity of dehydrogenase, phosphatase and urease

compared to the control. The highest increase in the enzymatic activity of dehydrogenase was obtained after the application of granulate B, and phosphatase and urease after the use of granulate D. The smallest increase in dehydrogenase and

phosphatase activity was obtained after the application of granulate C. Studies have shown that the highest soil activity of dehydrogenase was obtained after the application of dose 1, 2 and 3 of granulate B, phosphatases – after application of dose 1 of granulate D, and urease - when applying dose 2 of granulate D. Fertilization with increasing doses of granulates contributed to the increase in dehydrogenase, phosphatase and urease activity from date I to IV of soil collection from beneath rapeseed by 7.41%, 7.68% and 30.5%, respectively, compared to the control. Both the type of applied granulates A, B, C and D, increasing doses and the date of soil sampling had a significant impact on the increase in the activity of studied soil enzymes. The applied sludge-ash granulates stimulated the enzymatic activity of dehydrogenase, urease and phosphatases on all fertilizer objects. Studies have shown that there is a significant correlation between the activity of dehydrogenase vs. phosphatases and urease in the soil, to which sludge-ash granulates were applied at various doses and times.

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