Original Research

Enzymatic Activity of Soil after Applying Various Waste Organic Materials, Ash, and Mineral Fertilizers

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Abstract

Our study involved a 4-year pot investigation of loamy sand soil to which waste organic materials, brown coal ash, and mineral fertilizers containing nitrogen, phosphorus, potassium, and magnesium (NPKMg) were applied. Maize was the tested plant material grown in this soil mixture. The aim of this investigation was to determine the levels of enzymatic activities of acid phosphatase (ACP), alkaline phosphatase (ALP), urease (URE), and soil dehydrogenases (SDH) in this soil treated with organic materials, ash, and mineral fertilizers. Organic materials and ash significantly affected enzymatic activity of the soil. Mineral fertilization increased activities of ACP and SDH accompanied by a decrease in ALP activity. Data thus indicate that treatment of soils with organic materials, ash, and fertilizers alters soil enzymatic activity and, subsequently, the potential growth of corn.

Keywords: compost, enzymes, maize, waste-activated sludge, waste brown coal

Introduction

Soil nutrients, especial organic matter, are important drivers of soil microbial community composition [1]. Soil is a living dynamic system containing many free enzymes, immobilized extra-cellular enzymes, and enzymes within microbial cells. The soil enzymes include a wide spectrum of oxidoreductases, transferases, hydrolases, and lyases. Enzymes present in soil are similar to enzymes in other systems [2]. Accumulation of toxic compounds in soil is ecologically hazardous because of the risk that their remobilization may be delayed [3-6].

Soil enzymes play a critical role in catalyzing reactions leading to organic matter decomposition and serve as bioindicators of biochemical and microbial soil activity. The type of fertilization in plant cultivation affects enzymatic activity and thus the potential viability of plants to grow [7-9]. The species and long-term fertilization can result in microbial community shifts in soils [10, 11]. Many studies have proved that increasing soil organic matter content by additing organic manures can increase the biological activity in soil [12, 13]. Applied waste organic materials caused the increase of acidic and alkaline phosphatases activities [14]. According to Xie et al. [15], dehydrogenases activity is an indicator of soil quality and microbial activity.

However, the interaction between mineral fertilizers, which are currently employed in Polish agriculture and treated with organic matter on soil enzyme activities, is not known.

The aim of this investigation was thus to determine the viability of soil treated with both organic matter and fertilizers on various soil enzymes that serve as bioindicators of growth potential of crops. Corn grown in the treated soil serves as the crop and the enzymes ACP, ALP, URE, and SDH as soil enzymatic markers.

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Table 1. The scheme of the experiment.

I. Organic materials and ash (10% to the dry mass of soil material)	1. Control object (loamy sand)
	2. Brown coal from Konin mine
	3. Ash from Bełchatów
	4. Waste-activated sludge from Siedlce
	5. Waste-activated sludge from Łuków
	6. Farmyard manure
	7. Compost (waste-activated sludge from Siedlce + brown coal from Konin – 5:1)
	8. Compost (waste-activated sludge from Łuków + brown coal from Konin – 5:1)
	9. Compost (waste-activated sludge from Siedlce + ash from Bełchatów – 5:1)
	10. Compost (waste-activated sludge from Łuków + ash from Bełchatów – 5:1)
II. Mineral fertilization	1. 0 (without mineral fertilization)
	2. NPKMg (1:1:1:0.42)

Material and Methods

Soil Material

The soil samples for lab determinations were taken from pot soil of loamy sand composition. The pot experiment consisted of three replications over a 4-year period. Soil I included 10 fractions as shown in Table 1. The soil minerals added are also shown in Table 1. Maize was the crop tested (three plants per pot). During vegetation period (5 months) the moisture content was maintained at 60%. The soil material was dried, sieved through the screen of 1mm mesh and subjected to analyses. The soil was strongly acidic (pH in 1 mol $KCl\cdot dm^{-3}$ – pH 5.3); the organic carbon content was 9.2 $g\cdot kg^{-1}$ [16], total nitrogen 0.74 $g\cdot kg^{-1}$, mean content of available phosphorous 59.0 $mg\cdot kg^{-1}$, potassium 106.3 $mg\cdot kg^{-1}$, and magnesium 29.1 $mg\cdot kg^{-1}$.

Acid and Alkaline Phosphatase

The phosphatase activity determination method consisted of incubation of reactive mixture containing soil and a substrate. During the incubation process, release and accumulation of substrate hydrolysis products (ROH and HPO_4^{2-}) occurred [17]. The method of Tabatabai and Bremner [18] used sodium p-nitro-phenylophosphate dissolved in modified universal buffer (MUB) at pH 6.5 for acid (ACP) and at pH 11 for alkaline phosphatase (ALP), as a substrate. The enzymatic reaction was terminated using NaOH and $CaCl_2$. The color intensity (yellow) due to released p-nitrophenol was then measured spectrophotometrically.

Urease

Urease (URE) activity was determined colorimetrically following incubation of soil with urea and citrate buffer addition according to the modified method of Hoffmann and Teicher [19]. Before incubation, the toluene as bacteriostatic agent (completely inhibiting a microorganism's activity in soil) was added to prevent substrate intake, the formation of new enzymes, and microbial absorption of formed products.

Soil Dehydrogenases

Soil dehydrogenases (SDH) activity in soil was determined colorimetrically [20] using TTC (2,3,5-triphenylotetrasoline chloride) as a substrate, which was reduced to TPF (triphenyloformazane) during incubation. Soil acidity was adjusted using calcium carbonate. After incubation, the formazine was extracted from the soil with ethanol and measured colorimetrically. SDH activity was calculated based on the amount of formazine produced by a soil unit per time unit. All colorimetric determinations were determined with a Spectrofotometr UV-VIS Lambda 25 (Perkin Elmer).

Soil Organic Carbon

Organic carbon was recorded applying the oxidation-titrimetric method by Kalembasa [16]. In this method soil and potassium dichromate (0.083 $mol\cdot dm^{-3}$ and acid mixture: 5 parts by volume of concentrated sulphuric acid-1.84 sg AR and 1 part by volume of orthophophoric acid-1.7 sg AR was added. The mixture was heated in flash connected with a water radiator that held a stable temperature (161ºC) for 20 minutes. The temperature prevented the thermal decomposition of potassium dichromate. If the solution was green, that means the soil sample contained too much carbon in relation to the oxidation mixture. Assuming the colour was corrected, 3-4 drops of indicator (N-phenyloantranilic acid) was added and the suspension in the flask was titrated with 0.2 $mol\cdot dm^{-3}$ Mohr's salt-$(NH_4)_2Fe(SO_4)_2\cdot 6H_2O$. The colour of the solution should change from violet through blue to dark green. Calculation

Table 2. The value of pH in soil samples after ending the research (in 1 mol KCl·dm^{-3}).

Organic materials and ash	Mineral fertilization	
	-NPKMg	+NPKMg
1. Control object	5.4	5.7
2. Brown coal from Konin mine	5.4	5.6
3. Ash from Bełchatów	7.0	7.0
4. Waste-activated sludge from Siedlce	6.0	6.0
5. Waste-activated sludge from Łuków	5.0	5.6
6. Farmyard manure	6.3	6.1
7. Compost (waste-activated sludge from Siedlce + brown coal from Konin – 5:1)	5.7	5.6
8. Compost (waste-activated sludge from Łuków + brown coal from Konin – 5:1)	5.3	5.6
9. Compost (waste-activated sludge from Siedlce + ash from Bełchatów – 5:1)	6.5	6.5
10. Compost (waste-activated sludge from Łuków + ash from Bełchatów – 5:1)	6.3	6.2

of the soil organic carbon were based on the fact that 1 cm^3 of 0.083 mol·dm^{-3} potassium dichromate oxidizes 1.5 mg of carbon. It was assumed that C was the only reduced agent in the soil and that organic soil matter was formulated approximately as $Cn(H_2O)_m$.

Statistics

Results from chemical determinations were subjected to statistical analysis using analysis of variance (ANOVA) and significant differences determined by Tukey's test. The criterion for significance was set at $p<0.05$. In order to find the correlations between of organic carbon and activity of soil enzymes (ACP, ALP, SDH, URE).

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Biochemical Indicator of Soil Potential Fertility

Assessment of soil fertility growth potential based on a single enzyme may be biased and thus several enzymes were examined in the present study. Potential biochemical index of soil fertility (Mw) was calculated to include activites of ACP, ALP, URE, and SDH, as well as organic carbon content [21]:

$$Mw = (ACP + ALP + SDH + URE \times 10^{-1}) \times \%C$$

Results and Discussion

The changes of pH value in soil material after ending the experiment is presented in Table 2. The means of pH value (in 1 mol·dm^{-3} KCl) formed a reaction of researched soil samples in partition from very acid to indifferent. The highest pH value mean was in soil from object No. 3 (indifferent reaction) after ash application. The value pH on con-

Table 3. Acid phosphatase and alkaline phosphatase activity in kg D.M. (in mmol PNP·hr^{-1}).

Organic materials and ash (A)	ACP		ALP	
	Mineral fertilization (B)			
	NPKMg	+NPKMg	-NPKMg	+NPKMg
1*	1.00±0.05	0.06±0.01	0.20±0.01	0.18±0.01
2	1.12±0.05	1.38±0.10	0.36±0.01	0.42±0.03
3	0.37±0.01	0.42±0.01	0.26±0.01	0.29±0.02
4	1.04±0.01	1.26±0.01	0.35±0.01	0.31±0.01
5	1.30±0.03	1.23±0.01	0.26±0.01	0.23±0.01
6	0.72±0.03	1.08±0.01	0.41±0.01	0.30±0.01
7	1.13±0.03	1.42±0.01	0.32±0.01	0.29±0.01
8	1.16±0.01	1.24±0.01	0.28±0.02	0.28±0.03
9	0.67±0.03	0.99±0.03	0.42±0.01	0.35±0.01
10	0.61±0.03	0.95±0.05	0.29±0.01	0.32±0.01
$LSD_{0.05}$ for:				
Organic materials and ash (A)	0.11		0.04	
Mineral fertilization (B)	0.03		0.01	
Interaction (A) x (B)	0.15		0.06	
Interaction (B) x (A)	0.09		0.03	

*explanations as in Table 1

Table 4. Urease activity (in mg $N-NH_4 \cdot hr^{-1}$) and soil dehydrogenases activity in kg D.M. (in cm^3 $H_2 \cdot d^{-1}$).

Organic materials and ash (A)	URE		SDH	
	Mineral fertilization (B)			
	-NPKMg	+NPKMg	-NPKMg	+NPKMg
1*	10.61±0.23	9.75±0.23	1.33±0.09	1.08±0.09
2	7.66±0.23	7.83±0.23	3.27±0.29	3.83±0.38
3	11.25±0.24	10.25±0.17	1.79±0.05	1.70±0.16
4	11.16±0.06	10.03±0.03	1.66±0.16	2.91±0.10
5	10.75±0.17	9.00±0.23	1.58±0.09	1.83±0.16
6	11.08±0.05	10.23±0.26	1.45±0.05	2.70±0.05
7	8.92±0.02	10.65±0.17	2.00±0.16	2.24±0.10
8	9.75±0.17	10.88±0.09	1.16±0.19	1.74±0.19
9	8.00±0.23	9.08±0.09	0.83±0.16	1.12±0.14
10	8.50±0.23	11.11±0.13	1.29±0.05	1.16±0.07
$LSD_{0.05}$ for:				
organic materials and ash (A)	1.57		0.43	
mineral fertilization (B)	n.s.		0.11	
interaction (A) × (B)	2.22		0.60	
interaction (B) × (A)	1.31		0.36	

*explanations as in Table 1

stans horizon was in soils after the application of ash, sludges from Siedlce, and compost made of sewage from Siedlce + brown coal ash from Bełchatów. Positive correlations between soil enzymes and pH was observed by other authors. It is well-known that soil pH is a determinant for the composition of microbial communities, since completely different strains/species of microbes are predominant at different pHs [22, 23].

Acid and alkaline phosphatase activities were closely associated with soil acidity.

The acid phosphatase (ACP) activity was much higher than that of alkaline phosphatase (ALP) in all analyzed soil samples (Table 3). These enzyme activity levels in the soil significantly depended on the type of added organic material, ash, and NPKMg mineral nutrition. Considering the acid phosphatase activity in the soil with no mineral nutrition, its highest increase (by 0.30 mmol $PNP \cdot hr^{-1}$) in reference to control object (soil from object No. 5 with the addition of 10% sewage sludge from Łuków) was observed. The brown-coal ash addition resulted in the decrease of acid phosphatase activity by 63% as compared to the control. Statistical analysis revealed the dependence between organic carbon content in the soil with no mineral nutrition vs. acid phosphatase activity in that soil at the level of $r = 0.35$. Applying mineral nutrition for soil with organic additives caused the increase of acid phosphatase activity. The largest increase of acid phosphatase activity as compared to the control was recorded in soil from object No. 7, where two-month-old compost made of sewage sludge from Siedlce and brown coal from Konin along with NPKMg nutrition was used. The addition of organic materials and brown-coal ash, as well as NPKMg nutrition, significantly affected the enhancement of alkaline phosphatase activity in the soil in reference to the control. The largest increase was recorded in soil originating from object No. 9 (compost made of sewage sludge from Siedlce + brown coal ash) and from object No. 2 (brown coal from Konin + NPKMg). Dependence between organic carbon content in soil with organic additives and alkaline phosphatase activity ($r = 0.29$) as well as organic carbon content in the soil with organic additives + NPKMg vs. alkaline phosphatase activity ($r = 0.40$) was determined. Positive impact of waste organic materials application on activity of the enzyme was also reported by other scientists. Organic fertilization in most cases resulted in a statistically significant increase in the activity of alkaline and acid phosphatase in results achieved by Kalembasa and Kuziemska [24].

Adding the organic materials containing higher organic carbon amounts to the soil resulted in significant decrease of urease (URE) activity in reference to the control object (Table 4). Statistical analysis revealed considerable negative dependence between organic carbon content in the soil with organic additives and urease activity ($r = -0.67$ for $p<0.01$). Mineral nutrition did not significantly differentiate the urease activity level. Piotrowska [25] investigated spatial variability of total and mineral nitrogen content and

Table 5. Soil organic carbon (in %) and biochemical index of potential soil fertility (Mw).

Organic materials and ash (A)	C_{org}		Mw	
	Mineral fertilization (B)			
	-NPKMg	+NPKMg	-NPKMg	+NPKMg
1*	0.51±0.03	0.55±0.04	1.83±0.10	1.81±0.02
2	0.97±0.02	0.70±0.02	5.35±0.14	4.49±0.06
3	0.49±0.04	0.61±0.04	1.73±0.04	1.53±0.10
4	0.57±0.02	0.60±0.03	2.38 ±0.10	3.29±0.05
5	0.68±0.04	0.73±0.03	2.87±0.10	3.10±0.20
6	0.71±0.01	0.72±0.04	2.63±0.08	3.68±0.06
7	0.99±0.02	0.64±0.04	4.30±0.10	3.21±0.09
8	0.87±0.02	0.55±0.05	3.11±0.09	2.39±0.02
9	0.66±0.02	0.67±0.03	1.80±0.20	2.27±0.08
10	0.78±0.04	0.79±0.02	2.38 ±0.20	2.80±0.02
$LSD_{0.05}$ for:				
organic materials and ash (A)	0.06		0.22	
mineral fertilization (B)	0.02		n.s.	
interaction (A) × (B)	0.09		0.32	
interaction (B) × (A)	0.05		0.19	

*explanations as in Table 1

activities on the N-cycle enzymes in a luvisoil topsoil, proving that variation of coefficients (CV%) for total and mineral nitrogen forms was low for urease (UR).

Applied organic materials, brown-coal ash, and NPKMg nutrition considerably diversified soil dehydrogenase (SDH) activities. Mineral fertilizer additions to the soil enhanced the soil dehydrogenase activities. Significantly, the highest increase (about 3-fold) of dehydrogenases enzymatic activity in reference to the control object was recorded in the soil from object No. 2 (brown coal from Konin). The correlation coefficient between organic carbon content in the soil with organic materials addition and soil dehydrogenase activities for that soil amounted to r = 0.34. SDH is often used as an indicator of soil fertility and it also can denote the amount and activity of soil microbes [26]. Hence, higher SDH in OM (fertilization with organic manure) soil indicated that the long-term application of composted straw was more beneficial to microbial biomass and activity than the application of NPK and no fertilization [15]. The effect of spent engine oil on soil dehydrogenase activity was a progressive increase in the values obtained as the concentrations of the spent engine oil increased [27]. Fertilization with manure resulted in an increase of dehydrogenases and catalase activities in soil with increasing doses of manure [28].

Among studied organic materials, waste brown coal appeared to contribute the most to organic carbon content in the soil (Table 5). Mineral fertilization – on a background of organic materials – decreased C_{org} content in the soil. An indicator of potential biochemical soil fertility was in the present study (as similar as activities of particular enzymes) modified by a type of applied organic material and ash, as well as fertilization with Polimag 6 and ammonium nitrate. The highest increase of soil fertility was calculated in the object where waste brown coal from Konin was used (about 2.7-fold more in reference to the control). Results from the present study also indicate the connections between enzymatic activity in the soil with applied manure and mineral fertilization, making the increase of the fertility indicator in that object by almost 40%. The activity of dehydrogenases, urease and alkaline phosphatase in the soil with lupine cultivation was significantly higher than in the unsown soil [29].

The level of enzymatic activity of a soil determines its fertility, but also the ecological changes in the soil environment invoked by waste organic material application. Enhanced sorptiveness of sandy soil due to organic material application also increases their abundance in nutrients. Numerous publications also prove the increase of organic matter and water absorption capacity. Dehydrogenases are good indicators of the effects of contamination influence on biotic elements of the soil. Unlike chemical analyses results that give the information only on the contaminant contents, biological parameters reflect the environmental consequences, including those contaminants into the nutrition chain as well as their impact on enzymatic reactions in the soil [30]. Here, achieved high values of biochemical indicators of soil fertility after waste brown coal application indicate the great possibilities of its utilization for fertilizing. It was found that soil contamination with nickel had a negative impact on the activity of soil enzymes. The sensitivity of the analyzed enzymes to this heavy metal may be presented in the form of the following series: urease > dehydrogenases > alkaline phosphatase > catalase > arylsulphatase > β – glucosidase [21].

Conclusions

Applied organic materials and ash significantly differentiated the activity of acid phosphatase (ACP), alkaline phosphatase (ALP), urease (URE), and soil dehydrogenases (SDH). The increase of pH value of the soil material in particular objects considerably affected biological activity. Applied mineral fertilization on a background of organic materials and ash introduced into the soil caused the increase of acid phosphatase and dehydrogenases activities, while decreasing alkaline phosphatase activity. The biochemical index of soil fertility reached its highest value (5.35) after applying the waste brown coal at the rate of 10% of soil weight per pot.

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