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Response of soil phosphatases to glyphosate and its formulations – Roundup (laboratory conditions)

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ABSTRACT

This paper assesses the impact on certain phosphatase activities in soil of glyphosate and its formulations, i.e.: Roundup 360 SL and Roundup TransEnergy 450 SL, which contain various glyphosate salts (isopropylamine and potassium) and various surfactants (polyethoxylated tallow amine and polyethoxylated ether amine). The experiment was carried out on sandy loam samples with organic carbon content of 10.9 g/kg. Aqueous solutions of pure glyphosate and its formulations were added to the soil. The amounts of applied glyphosate and its salts were: 1, 10 and 100 mg/kg. On days 1, 7, 14, 28 and 56 the activity of alkaline phosphomonoesterase (ALP); acid phosphomonoesterase (ACP); phosphodiesterase (PD); phosphotriesterase (PT) was measured spectrophotometrically. The effect of glyphosate and its formulations depended on the herbicide dosage and day of experiment. ALP and PD were the most susceptible to the presence of glyphosate. A comparison of the impact of glyphosate and its formulations showed that Roundup 360 SL was the most toxic. This could have resulted from the presence of surfactant polyethoxylated tallow amine in formulation. The correlation coefficients and principal component analysis indicated a significant positive relationship between the phosphatase activities in soil containing glyphosate. Significant correlation at $P < 0.01$ was noted among ALP and ACP, and among ALP and PD. Additionally, at $P < 0.05$, PD was significantly correlated with ACP, and PT with ALP and with PD.

Keywords: decomposition; soil enzymes; phosphorus metabolism

Glyphosate [N-phosphonomethyl]glycine is a systematic, non-selective, organophosphorus herbicide used worldwide in agriculture and industrial zones (Al Rajab and Hakami 2014). It is an acid, but it is commonly used in salt form: isopropylamine, ammonium and potassium (Płatkowski and Telesiński 2015b). The herbicidal effects of glyphosate are due to the inhibition of the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), an enzyme from the shikimate pathway, which leads to prevention of the biosynthesis of the amino acids: phenylalanine, tyrosine, and tryptophan (Gomes et al. 2014). Glyphosate is applied foliarly, but a significant amount of this herbicide may reach the soil (Lane et al. 2012). Glyphosate in soil readily binds to clay minerals and hydrous oxides through its phosphoric acid moiety (Haney et al. 2000) and its adsorption rates correlate with phosphate sorption sites. It binds tightly to the soil particles, reaching a persistence of up to 170 days and a usual half-life of 45–60 days (Vereecken 2005). It is generally assumed that the most significant route of glyphosate degradation is decomposition of the C-P bond catalysed by microbial enzymes (C-P lyase, phosphatases) (Forlani et al. 1999). The final product of decomposition should be the orthophosphate ion PO_4^{3-}, as well as organic byproducts that do not depend on the mechanism of decomposition (Sudoł and Krzyśko-Łupicka 2005).

The name phosphatase describes a group of enzymes that hydrolyses esters as well as anhydrides of phosphoric acid. There are different phosphatases in soils: phosphomonoesterase, phosphodiesterase, phosphotriesterase, phospholipase, inorganic pyrophosphatase and enzymes acting on P-N-bonds (Wang et al. 2011).

This paper describes the effects in sandy loam of different forms of glyphosate and its formulation (Roundup 360 SL and Roundup 450 TransEnergy SL)

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on the activities of alkaline phosphomonoesterase (ALP); acid phosphomonoesterase (ACP); phosphodiesterase (PD) and phosphotriesterase (PT).

MATERIAL AND METHODS

In the experiment, three forms of glyphosate were used: pure glyphosate, isopropylamine salt and potassium salt. Pure glyphosate (analytical standard 99.7% purity) was purchased from Fluka (Germany). Isopropylamine and potassium salts of glyphosate were used, respectively, as Roundup 360 SL (360 g/dm^3) and Roundup TransEnergy 450 SL (450 g/dm^3) produced by Monsanto Company. Glyphosate formulations also contained surfactants: polyethoxylated tallow amine (Roundup 360 SL), and polyethoxylated ether amine (Roundup TransEnergy 450 SL).

Soil samples were collected from the Gumieniecka Plain (53°24'N, 14°28'E), located in the West Pomeranian District, Poland. This field remained under conventional farming practices and had no history of glyphosate application in the previous three years. Soil samples were taken from 0–20 cm of the bulk soil on the field.

The soil was classified as sandy loam (43% fraction 1.0–0.1 mm; 30% fraction 0.1–0.02 mm; 27% fraction < 0.02 mm). Selected physical and chemical characteristics of the soil are as follows: pH 6.8, organic carbon (C) 10.9 g/kg, total nitroegn (N) 1.39 g/kg. To consider the spatial heterogeneity, soil samples were taken in triplicates, and each replicate consisted of five 10-cm auger cores. Then, the soil samples were manually and gently crumbled, mixed thoroughly, air dried at room temperature, and sieved through a 2-mm mesh to remove stones and plant roots before being used.

The experiments were conducted with the following 10 treatments: 0 (solvent control), and 1, 10, 100 mg/kg for glyphosate, its isopropylamine and potassium salts. Each treatment was replicated three times at each sampling stage. For each treatment, 1 kg soil was thoroughly mixed with glyphosate stock water solution to achieve the setting concentration. Samples were adjusted to 60% maximum water holding capacity.

All treatments were incubated in the dark at 20°C for 56 days in hermetic polyethylene bags. Samples from each treatment were collected after 1, 7, 14, 28 and 56 days for assays of phosphatase activities.

The acid phosphomonoesterase (EC 3.1.3.2) and alkaline phosphomonoesterase (EC 3.1.3.1) activities were determined as described by Tabatabai and Bremner (1969). The phosphodiesterase (EC 3.1.4.1) and phosphotriesterase (EC 3.1.8.1) activities were determined according to Browman and Tabatabai (1978), and Eivazi and Tabatabai (1977), respectively. The yellow-band absorbance of the filtrate due to ρ-nitrophenol was measured at 400 nm with a Shimadzu UV-1800 spectrophotometer (Kyoto, Japan). Enzyme activities were calculated using a calibration curve.

Phosphatase activity determinations were performed on three replicates for each treatment, and the significance of the observed differences was verified using a two-way analysis of variance followed by the post-hoc Tukey's *HSD* test. Differences with a P value of < 0.05 were considered as significant. Principal component analysis (PCA) was used to analyse data for phosphatase activities. The first two principal components (PC1 and PC2) were selected for further interpretation of the results. The correlation coefficients between the measured parameters were interpreted as ordinary correlation coefficients. All statistical analyses were carried out using the Statistica 10.1 program (StatSoft, Kraków, Poland).

Mean values of the enzyme activities were also used to calculate the relative changes according to the formula defined by Chaer et al. (2009):

$$RCh = \left(\frac{T}{C} - 1\right) \times 100\%$$

Where: T – mean enzyme activity in the treated soil sample; C – mean value obtained for the control.

RESULTS AND DISCUSSION

The effect of glyphosate and its formulations, on the activity of soil acid phosphomonoesterase; alkaline phosphomonoesterase; phosphodiesterase and phosphotriesterase is presented on Figures 1–4.

In soil untreated with glyphosate and its formulations activities of phosphatases were ranged: ACP – from 96.41 to 124.39 mg p-NP/kg dry matter (DM)/h (Figure 1), ALP – from 310.06 to 355.13 mg ρ-NP/kg DM/h (Figure 2), PD – from 5.84 to 7.42 mg p-NP/kg DM/h (Figure 3) and PT – from 14.61 to 16.19 mg p-NP/kg DM/h (Figure 4).

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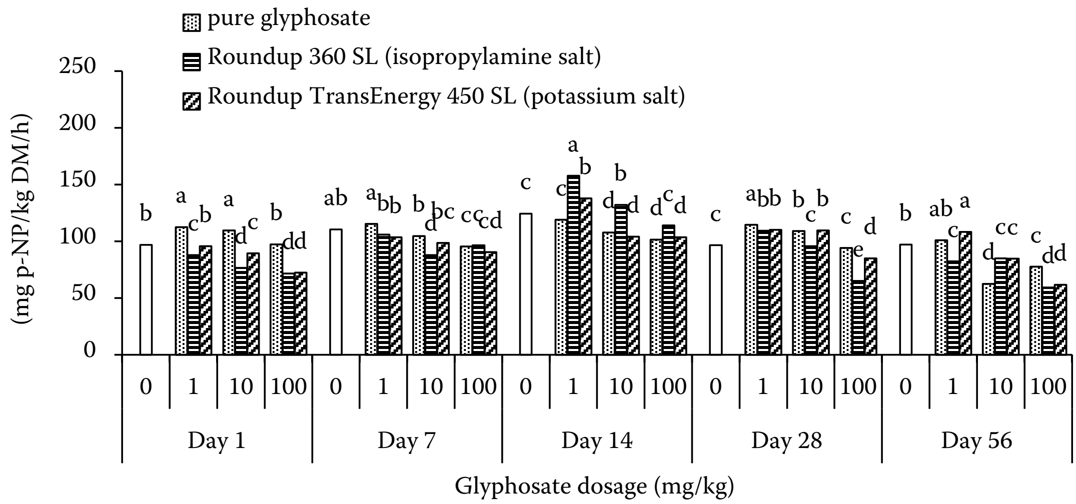

Figure 1. Acid phosphomonoesterase activity in soil treated with glyphosate and its formulations. Values denoted with the same letters within the day of experiment do not differ statistically ($P < 0.05$); p-NP – p-nitrophenol; DM – dry matter

Compared with the control, treatment with pure glyphosate and its formulations caused different changes (stimulation or inhibition) of phosphatase activities. The impact of glyphosate and its formulations depends on the day of the experiment and herbicide dosages. These results are in agreement with our previous studies (Płatkowski and Telesiński 2015a,b). The effect of glyphosate on soil enzymes involved in phosphorus metabolism gave conflicting results: inhibition (Sannino and Gianfreda 2001, Floch et al. 2011, Yu et al. 2011) and stimulation (Nakatani et al. 2014, Cherni et al. 2015). Tejada (2009) reported that the effect of glyphosate on phosphatases is higher in sandy soil than in clay soil. The results are in agreement with our previous study (Płatkowski and Telesiński 2015b). According to Franz et al. (1997) the phosphate group in glyphosate readily adsorbs to clay, aluminium and iron oxides in soil. However Krzyśko-Łupicka and Sudoł (2008) have shown that glyphosate does not accumulate in soil, since microorganisms are able to breakdown C-P bonds, resulting in the release of inorganic phosphorus. Microbial degradation of glyphosate, and other

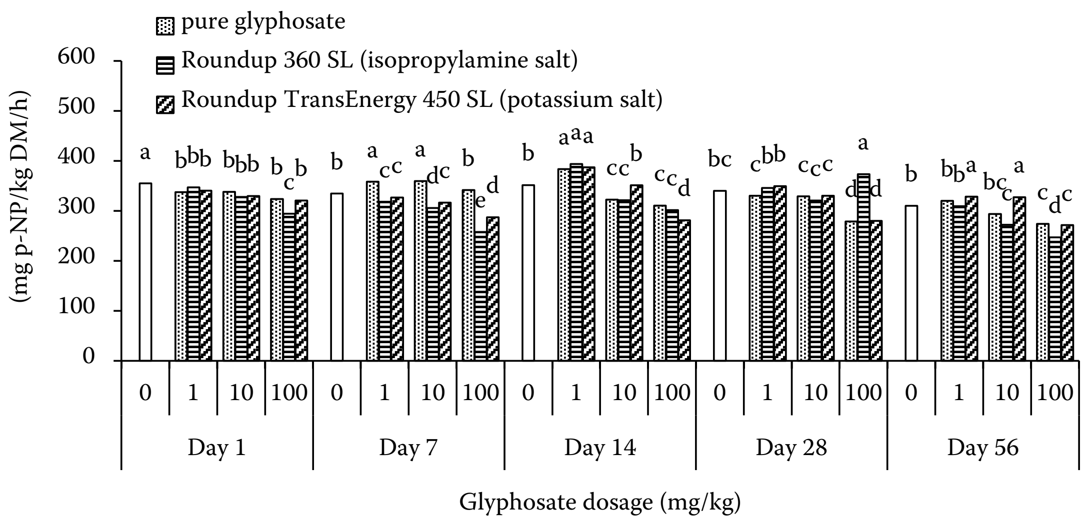

Figure 2. Alkaline phosphomonoesterase activity in soil treated with glyphosate and its formulations. Values denoted with the same letters within the day of experiment do not differ statistically ($P < 0.05$); p-NP – p-nitrophenol; DM – dry matter

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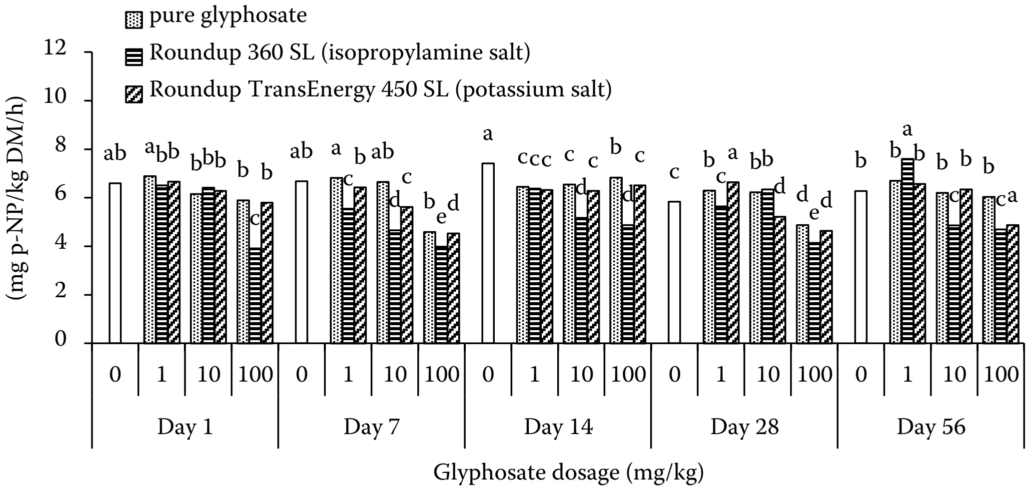

Figure 3. Phosphodiesterase activity in soil treated with glyphosate and its formulations. Values denoted with the same letters within the day of experiment do not differ statistically ($P < 0.05$); p-NP – p-nitrophenol; DM – dry matter

herbicides, in soils there is a function of three key variables: the ability of the microorganisms to degrade the pesticides, the quantity of these microorganisms in the soil, and the activity of the soil microbial enzyme system (Sannino and Gianfreda 2001). Glyphosate with a P-containing amino acid could be a P-source, and provide C and N for soil microorganisms, when added to soil (Lane et al. 2012). Hence, Yu et al. (2011) reported that soil acid phosphomonoesterase activity could be used as a biochemical indicator of glyphosate breakdown.

Analysis of the mean relative changes of the measured phosphatase activities showed that the application of glyphosate and its salts at dosages of 1 mg/kg and 10 mg/kg caused a slight impact on all enzymes. Only after the application of glyphosate isopropylamine salt (Roundup 360 SL) at a dosage of 10 mg/kg the decrease more than 10% was noted in phosphodiesterase activity. In soil treated with pure glyphosate and its salts at a dosage of 100 mg/kg a decrease in all mean phosphatase activities was observed. The acid phosphomonoesterase and phosphodiesterase proved to be the most sensi-

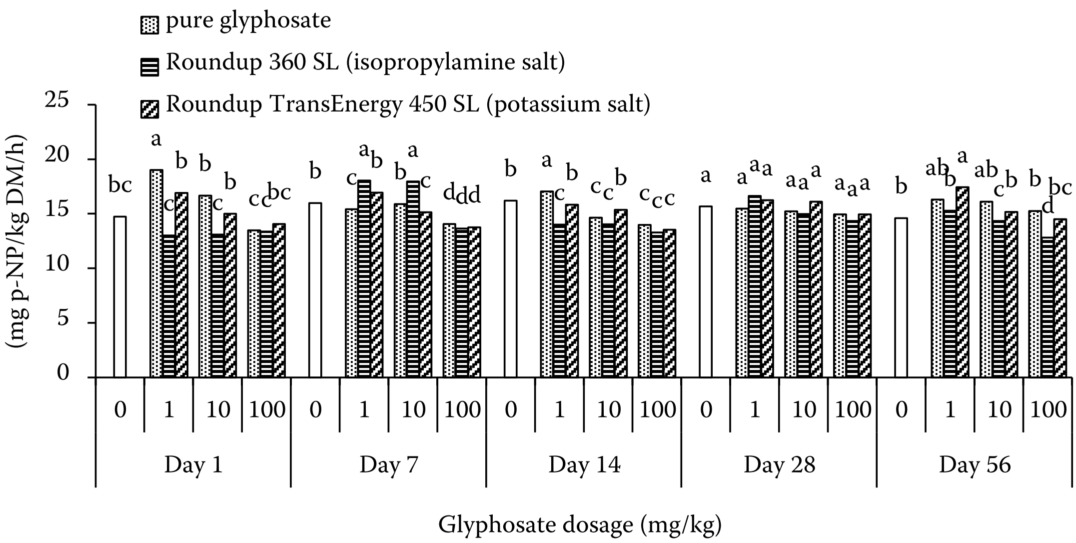

Figure 4. Phosphotriesterase activity in soil treated with glyphosate and its formulations. Values denoted with the same letters within the day of experiment do not differ statistically ($P < 0.05$); p-NP – p-nitrophenol; DM – dry matter

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Table 1. Mean relative changes of phosphatase activities (%) in soil treated with glyphosate and its formulations

Enzyme	Glyphosate dosage (mg/kg)								
	1	10	100	1	10	100	1	10	100
	pure glyphosate			Roundup 360 SL (isopropylamine salt of glyphosate)			Roundup TransEnergy 450 SL (potassium salt of glyphosate)		
ACP	7.24	−5.95	−11.19	3.49	−9.00	−22.54	4.70	−7.28	−21.34
ALP	2.28	−2.81	−9.57	−1.27	−8.45	−18.54	2.49	−2.06	−14.82
PD	1.09	−3.09	−13.91	−3.39	−16.26	−34.09	−0.51	−9.27	−19.68
PT	7.82	1.80	−7.02	−0.23	−3.60	−12.55	8.04	−0.46	−8.22

ACP – acid phosphomonoesterase; ALP – alkaline phosphomonoesterase, PD – phosphodiesterase; PT – phosphotriesterase

tive to the presence of glyphosate (Table 1). This is similar to our previous research in sandy soil (Płatkowski and Telesiński 2015b). The greatest effect on phosphatase activities in soil was observed after the application of Roundup 360 SL (containing glyphosate isopropylamine salt), but the presence of polyethoxylated tallow amine causes glyphosate toxicity increase. Several studies have reported that the toxicity glyphosate-based herbicides to organisms is largely due to surfactants in the mixture. Specifically, glyphosate formulations containing polyethoxylated tallow amine were more toxic than pure active substance or some formulations with other surfactants (Howe et al. 2004, Moore et al. 2012, Uren-Webster et al. 2014). However, the results obtained by Sihtmäe et al. (2013) show the different effect of two glyphosate formulations (Roundup Quick and Roundup Max) on the soil bacteria that are the main biological detoxifiers of glyphosate in soil. Roundup Quick (without polyethoxylated tallow amine) was less toxic to soil bacteria strains than Roundup Max (with polyethoxylated tallow amine).

PCA was carried out to ascertain the relationships between the activity of phosphatases in soil. Eigenvalues from PCA indicated that the first two principal components accounted for 78.89% of the variance of data (PC1: 59.46% and PC2: 19.43%; Table 2 and Figure 5) ACP, ALP and PD had a major positive effect on PC1, while PT had a major positive effect on PC2.

Correlation analysis among soil phosphatase activities in soil treated with glyphosate and its formulations indicated that acid phosphomonoesterase and phosphotriesterase were not sig-

Table 2. Pearson product-moment correlation coefficients between activities of phosphatases in soil treated with glyphosate and its formulations, and scores of the PC1 and PC2, which explained 52.46% and 19.43% of the variance in the PCA analysis, respectively

Component	ACP	ALP	PD	PT
PC1	0.791	0.896	0.768	0.599
PC2	−0.381	−0.227	0.064	0.759

ACP – acid phosphomonoesterase; ALP – alkaline phosphomonoesterase; PD – phosphodiesterase; PT – phosphotriesterase

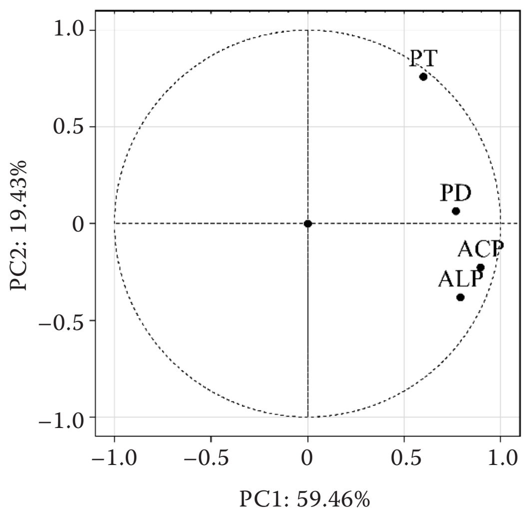

Figure 5. Principal component analysis for activity of phosphatases in soil treated with glyphosate and its formulations. ACP – acid phosphomonoesterase; ALP – alkaline phosphomonoesterase; PD – phosphodiesterase; PT – phosphotriesterase

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Table 3. Correlation coefficients (Pearson product-moment) between the activity of phosphatase in soil treated with glyphosate and its formulations

	ACP	ALP	PD	PT
Acid phosphomonoesterase	1.000	–	–	–
Alkaline phosphomonoesterase	0.696**	1.000	–	–
Phosphodiesterase	0.384*	0.605**	1.000	–
Phosphotriesterase	0.287	0.365*	0.354*	1.000

*$P < 0.05$; **$P < 0.01$

nificantly correlated (Table 5). Other phosphatase activities were significantly positively correlated ($P < 0.05$). Moreover, alkaline phosphomonoesterase had a significant positive correlation with acid phosphomonoesterase (0.696, $P < 0.01$) and with phosphodiesterase (0.605, $P < 0.01$) (Table 3).

In conclusion, the effect of glyphosate and its formulations depend on the day of the experiment and herbicide dosages. In soil treated with all glyphosate forms at dosages of 1 and 10 mg/kg, slight changes of mean phosphatase activities in sandy loam were observed, whereas the glyphosate dosage of 100 mg/kg decreased the mean phosphatase activities. Among the measured phosphatases, alkaline phosphomonoesterase and phosphodiesterase occurred the most vulnerable on the presence of glyphosate and its formulations in soil. The highest inhibitory effect on phosphatase activities in soil was observed after the application of Roundup 360 SL (containing glyphosate isopropylamine salt and surfactant polyethoxylated tallow amine). Application of the highest dosage of Roundup 360 SL caused a decrease in mean ALP, ACP, PD and TP at the level: 22.54, 18.54, 34.09 and 12.55%, respectively, whereas in soil treated with pure glyphosate the inhibition of mean phosphatase activities was 11.19, 9.57, 13.91 and 7.02%, and in soil wit Roundup TransEnergy 450 SL (containing glyphosate potassium salt and surfactant polyethoxylated ether amine) – 21.34, 14.82, 19.68 and 8.22%, respectively.

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