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Influence of pesticide (glyphosate) on dehydrogenase activity, pH, Eh and gases production in soil (laboratory conditions)

R.P. Bennicelli, A. Szafranek-Nakonieczna, A. Wolińska, Z. Stępniewska, and M. Bogudzińska*

John Paul II Catholic University of Lublin, Department of Biochemistry and Environmental Chemistry, Kraśnicka 102, 20-718 Lublin, Poland

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A b s t r a c t. Enzyme activity is a sensitive indicator of fertility, metabolic processes and circulation of matter as well as soil environment contamination. Dehydrogenases (EC 1.1.1.1) are the kind of enzymes belonging to oxidoreductases class, which catalyzes oxidation and reduction reaction. Their activity give information on soil microflora oxidative activity and intensive matabolism of soil microorganisms. Heavy metals, PAH's or pesticides decrease the soil dehydrogenase activity. Glyphosate is the most common herbicide used worldwide and as other pesticides is toxic for living organisms. The aim of the study was to determinate dehydrogenase activity (DHA) in the soils enriched with glyphosate (1 μg and 10 μg g^{-1} of pesticide doses) during time (42 days), under laboratory conditions at temperature 20°C. The decrease of DHA activity was observed that depended on the pesticide dose.

K e y w o r d s: dehydrogenase, glyphosate, oxydoreduction potential, gas formation

INTRODUCTION

Glyphosate is a broad-spectrum, non-selective, systemic and post-emergence herbicide widely used in soil cultivation, forestry, rights-of-way and aquatic systems to prevent grass and weeds competition with plant seedlings. At low doses it is used as a plant growth regulator. Glyphosate (structural formula in Fig. 1) is often used as a pesticide component., For example, the major product Roundup®, which is formulated as 41% of the glyphosate in form of isopropylamina salt and 59% inert ingredients, and in that connection is more toxic than the glyphosate alone. It is a broad-spectrum, non-selective, systemic and post-emergence herbicide widely used in soil cultivation, forestry, rights-of-way and aquatic systems to prevent grass and weeds competition with plant seedlings. At low doses it is used as a plant growth regulator. Glyphosate is a polar substance that is highly soluble in water (12 g l^{-1} at 25°C), and insoluble in most organic solvents. In soil is moderately persistent; its half-life is reported between 1 to 174 days. Glyphosate in soil is transformed to aminomethylphosphonic acid (AMPA), which is non-persistent metabolite. As a effect of mentioned transformations and in presence of dehydrogenase (microorganisms) glyphosate give CO_2 and H_2O (Forlani *et al.,* 1999). Glyphosate degradation in soil is mainly the reason of microbial activity, while the chemical decomposition and photolysis play a minor role. Most soils strongly absorb glyphosate on silicates layer, metal oxides, non-crystalline material and organic matter (Veiga *et al.,* 2001). Genetically modified plants have been designed to tolerate glyphosate. Its residue may enter into food chain and is found as a contaminant in rivers. Manufacturer-recommended doses are considered as a safe for environment (Pandey and Singh, 2006; Richard *et al.,* 2005).

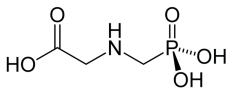

Fig. 1. Structural formula of glyphosate.

Glyphosate acid and its salts are moderately toxic compounds in EPA toxicity class II. Labels of products containing these compounds must bear the signal word 'warning' (Kamrin, 2000). Usage of pesticide in agriculture have caused several serious soil health problems. Pesticide can inhibit biochemical reactions in soil environment.

*Corresponding author's e-mail: szafik@kul.lublin.pl

Dehydrogenase activity in soil is a result of the activity different dehydrogenases, which are an important factors confirming the presence of living organisms (dehydrogenase exist in soil when microorganisms cells are intact), and often is considered as a very sensitive index of biological condition of soil (Włodarczyk *et al.,* 2005). Dehydrogenases play an essential role in the initial stage of the oxidation of the soil organic matter by transferring hydrogen or electrons from substrates to acceptors and may be used as a measurement of overal microbial activity (Pandey and Singh, 2006).

Glyphosate as an organophosphonate can be used as a source of P, C or N by either gram-positive as gram-negative bacteria (Zabaloy *et al.,* 2008).

Literature, until now give rather little informations about influence of different pesticides on emissions of greenhouse gases, such as CO_2, N_2O or CH_4. Concentrations of these gases have increased in the last few decades (Rastogi *et al.,* 2002).

Carbon dioxide is an important gas responsible for 60% of the total greenhouse effect. CO_2 is released from the soil as result of three types of biological processes: microbial, root and faunal respiration. Soil is the major source of atmospheric CO_2. There are several factors influencing its production in soil, such as: texture, moisture, pH and salinity. Mentioned factors effect on soil microbial activity and by root respiration influence on CO_2 production (Rastogi *et al.,* 2002).

Nitrous oxide is atmospheric trace gas and contributes about 6% to the greenhouse effect. Its long atmospheric residence time (about 114 years) and chemical activity both in trophosphere and in stratosphere cause its high potential to directly or indirectly influence global climate. It is 310 times a more potent greenhouse gas then CO_2 on 100 years timescale (Krithika *et al.,* 2008).

The purpose of the current study was to research the influence of glyphosate on soil dehydrogenase activity (DHA) and on soil gases (CO_2,N_2O) emission in the Mollic Gleysols, Eutric Fluvisols and Terric Histosols taken from surface layer (0-20 cm).

MATERIALS AND METHODS

The laboratory experiment was performed on 3 types of soils: Mollic Gleysols, Eutric Fluvisols and Terric Histosols, differing each other by ther origin. The relevant characteristics of the soil material are reported in Table 1.

Mollic Gleysols (comes from Wieprz river valley), are semihydrogenic soils, formed on rich in organic matter and calcium carbonate parent material under long-term influence of ground water or flooding by periodically stagnant water from rainfalls and collected over the non pervious layer.

Eutric Fluvisols (taken from Vistula river valley), belong to the group of alluvial soils created under the influence of erosional-sedimentational action of surface water.

Terric Histosols (taken from Bystrzyca river valley are hydrogenic soils that arise from peat soils as a effect of dehydratation which causes intensive organic matter mineralization.

The soil samples were taken from Ap-level (0-20 cm) and examined in 3 combinations with different doses of pesticide. Soil samples were placed in dark bottles and enriched with the glyphosate as follows: with 1 μg (first combination) and 10 μg (next version) and 0 μg (control) of pesticide per 1g of soil. Thus prepared samples (8 bottles for each combinations from every investigated soils) were incubated in thermostatic chamber at 20ºC. After 3 days, and then each 7 days interval till to 42 day,s the dehydrogenase activity (DHA), redox potential (Eh), pH values and gases composition in a head space of soil were determined.

Method for measuring soil dehydrogenase activity required an addition of artificial electron acceptor TTC (3% solution of 2,3,5-triphenyltetrazolium chloride) to soil samples. TTC is reduced to formazane, which is extracted by ethanol (95%) and give TPF (triphenyloformazan). Formazan concentration was estimated by spectrophotometer HITACHI UV-VIS U-2001 at λ=485 nm (Casida, 1977).

Redox potential (Eh) and pH were controlled by using Eh and pH-meter Pionneer 65 (Radiometer Analytical S.A.) and adequate electrodes.

T a b l e 1. Characteristic of the soil material (upper level, 0-20 cm)

Properties	Diameter (mm)	Terric Histosols	Eutric Fluvisols	Mollic Gleysols
Grain size distribution (%)	1-0.1	n.a.	15	38
	0.1-0.02	n.a.	46	25
	<0.025	n.a.	39	37
$C_{org.}$ (%, mass)		36.5	2.47	2.12
pH (in H_2O)		7.5	6.3	7.5
Humidity (%, mass)		37.5	1.8	2.0

n.a. – not analysed.

Gas composition (CO_2 and N_2O) in head space was analysed chromatographically (Varian CP-3800, equipped with TCD and ECD detectors). All gas and enzyme analyses were performed in triplicate and the reported values are averages.

RESULTS AND DISCUSSION

Preliminary analysis of dehydrogenase activity (control) showed that the highest activity was observed in Mollic Gleysols, where average concentration TPF was equal to 70 μg g^{-1} min^{-1} and was over 84% more than in Eutric Fluvisols and over 80% than in Terric Histosols, what are presented in Fig. 2.

Both 1 and 10 μg of glyphosate addition to soils caused a decrease of DHA activity dependent on the doses (Figs 2, 3). The strongest effect of glyphosate was observed in Terric Histosols (10 μg g^{-1} of soil), where reduction of DHA activity by 80% relative to control soils (non amended with Glyphosate) was noted (Fig. 2a). The most resistant to glyphosate was Eutric Fluvisols, in 10 μg g^{-1} of soil dose, where DHA activity dropped by 24% (Fig. 2b). Inhibition effect was not confirmed in Brazilian soil tested by Andreá *et al.* (2003), where dehydrogenase activity was slight higher than at the beginning of the experiment after month from glyphosate application. In that case authors suggested, that glyphosate stimulated DHA activity, which means that the herbicide might stimulate the soil oxidative processes. Conversely, in typical Ardiudoll from Argentina reduction of DHA for about 48% in comparison to control sample was observed (Zabaloy *et al*., 2008). Sannino and Gianfreda (2001) confirmed that glyphosate can inhibit soil phosphatase activity. In presence of glyphosate they found the drop of enzyme activity from 5 to 98%. The effect of glyphosate on the carbon dioxide concentration in soils headspace during incubation period is shown in Fig. 4a, b, and c. At the beginning of the experiment concentration of CO_2 varied from 0.11 to 0.96% and increased up to 12% at the end. In all cases in control samples an increase of CO_2 was gradual and reached the maximum between 35th and 42nd days of incubation. In Terric Histosols and Eutric Fluvisols, inversely than in Mollic Gleysols, in combination with addition of pesticide, the concentration of CO_2 was significantly lower than without it. The biggest differences were registered in Terric Histosols between 3rd and 21st day. Then CO_2 did not exceed 2%, whereas from 28th days reached up to 4% and maintained at this level up to the end of the experiment time with both doses of glyphosate. Meanwhile, the level of CO_2 exceeded 10% in the control at the last incubation week. In Eutric Fluvisols, differences in CO_2 concentration were observed after 7th day of incubation and at each week were more significant between particular doses. Value of CO_2 similar to those reported in Terric Histosols was at the lowest level in soil with tenfold doses of glyphosate. On the contrary, in Mollic Gleysols, between 3rd and 14th days of

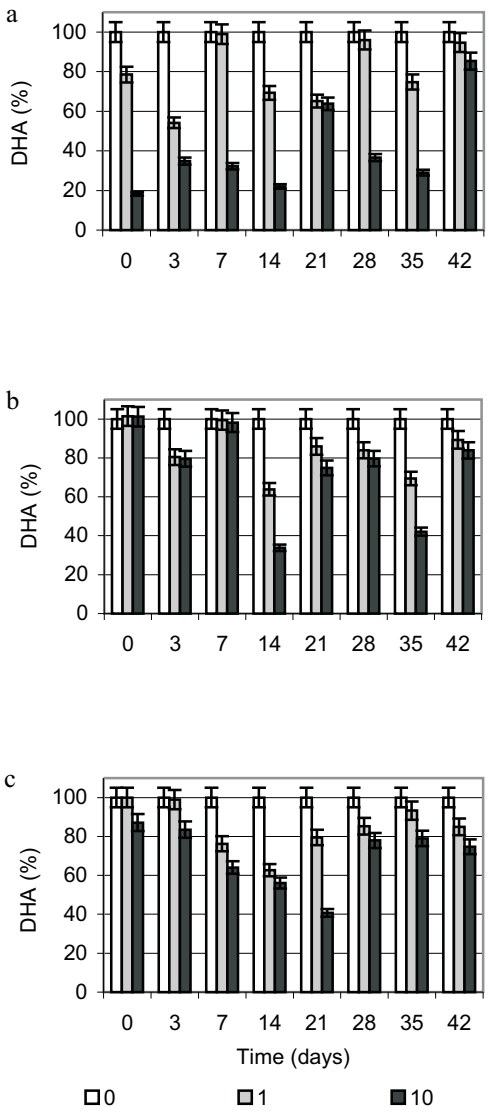

Fig. 2. Dehydrogenase activity (DHA) in: a – Terric Histosols, b – Eutric Fluvisols, c – Mollic Gleysols treated with glyphosate doses: 0 – control, 1-1 μg g^{-1}, 10-10 μg g^{-1}, with respect to the control (% of activity).

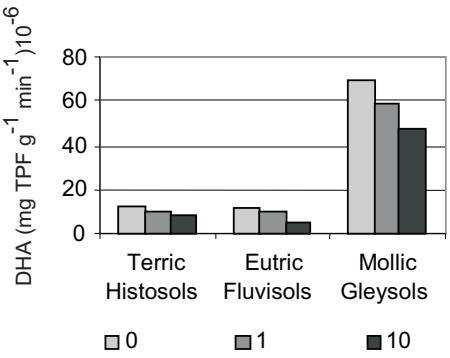

Fig. 3. Mean dehydrogenase activity (DHA) in: Terric Histosols, Eutric Fluvisols, and Mollic Gleysols, in 3 combinations of glyphosate doses: 0 – control, 1-1 μg g^{-1}, 10-10 μg g^{-1}.

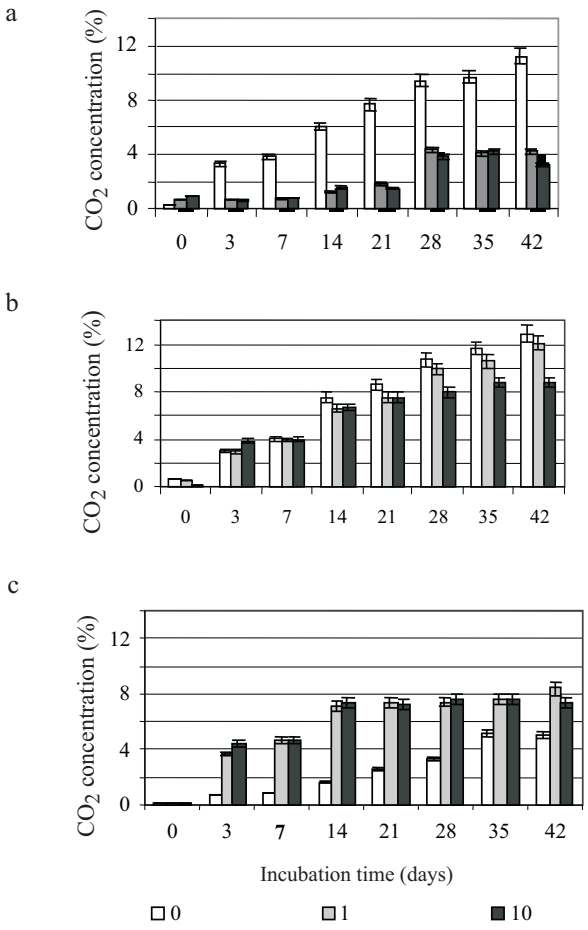

Fig. 4. Changes in CO_2 concentration (%) during incubation time in: a – Terric Histosols, b – Eutric Fluvisols, c – Mollic Gleysols for particular glyphosate doses: 0 – control, 1-1 μg g^{-1}, 10-10 μg g^{-1}.

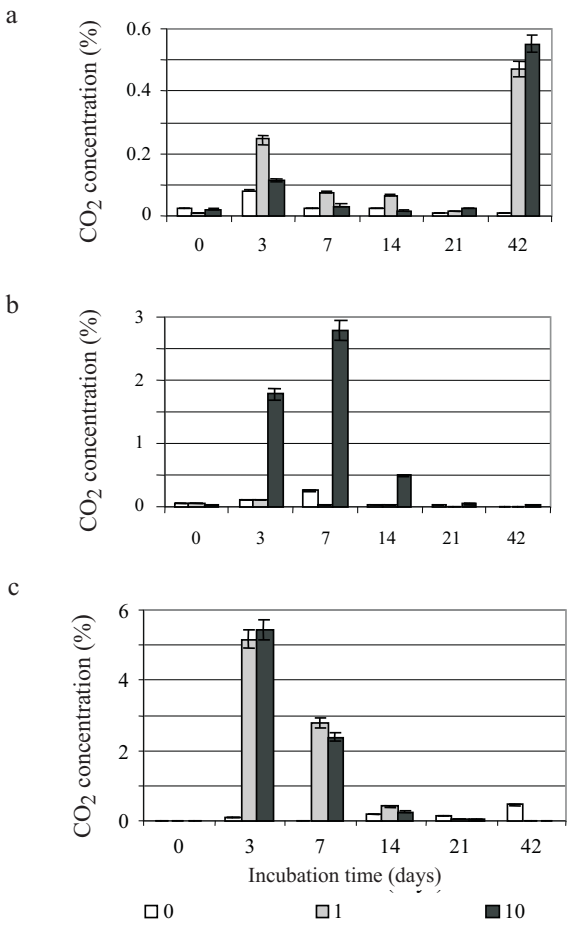

Fig. 5. Changes in N_2O concentration (%) during incubation time in:a – Terric Histosols, b – Eutric Fluvisols, c – Mollic Gleysols for particular glyphosate doses: 0 – control, 1-1 μg g^{-1}, 10-10 μg g^{-1}.

experiment, the concentration of CO_2 increased above 7% and up to 42nd maintained about 8%, while it slowly increased to 5% in the control sample to the end of the laboratory test. Similar experiment was performed with use of Fonofos, where both single as tenfold doses resulted in decrease of CO_2 formation for 10.2 and 62%, proportionally to dose applied (Stępniewska *et al.,* 2008). It is also well known, that temperature has a marked effect on CO_2 production and summer temperature favoured CO_2 formation (Rastogi *et al.,* 2002).

The concentration of nitrous oxide in the headspace differed among soils, but in all investigated soils reached the highest values at single and tenfold dose of glyphosate. The highest values were detected in Mollic Gleysols (both single as tenfold doses) at 3rd day of incubation and reached 5.2 and 5.4 (%, vol.), respectively (Fig. 5c). That dimension exceed 56 times from the value obtained in control, and was the highest among all tested soils. In the case of Eutric Fluvisols a significant increase was observed after 3rd day with maximum value 2.8 (%, vol.) in 7th day of the experiment. In Terric Histosols, formation of nitrous oxide was the lowest in comparison to the rest of examined soils. The first peak was found in 3rd day, similar as in Eutric Fluvisols and Mollic Gleysols, but it was considerably lower (Fig. 5a, b, and c). The second and also the highest peak was noted at 42nd day of experiment and exceed 0.5 (%, vol.). In all examinated soils an increase of N_2O concentration was observed after 3rd day of incubation. Maximum values of N_2O in Terric Histosols, Eutric Fluvisols and Mollic Gleysols were observed at different times, on 42nd, 7th and 3rd, respectively. In the case of Terric Histosols and Eutric Fluvisols the highest concentration was observed in presence of both single and tenfold supplement of glyphosate.

The differences at N_2O concentration in all treatments were not great and usually not exceeded 0.5 (%, vol.). Stronger influence of tenfold dose of glyphosate rather than single dose was observed in Eutric Fluvisols. In combination with single dose nitrous oxide was at the same level as in the control sample. Kinney *et al*. (2005) found that some pesticide like mancozeb, chlorothalonil, prosulfuron inhibited N_2O

production. Other autors stated that Fonofos also mitigated N_2O formation (Stępniewska *et al*., 2008). In the case of glyphosate in all investigated soils N_2O concentration increased with growth of pesticide dose.

Statistical analysis were performed between DHA activity and: CO_2 concentration, Eh potential, pH values in all investigated soils (with and without glyphosate), what resulted in estimating high R^2 ratio values (Table 2). In all tested soils CO_2 concentration were correlated with DHA activity, especially in a treatment without glyphosate. With single and tenfold doses of glyphosate correlation also existed but had lower R^2 ratio.

During experiment time Eh value decreased in all tested soils, while pH dropped in Terric Histosols and Mollic Gleysols, but increased in Eutric Fluvisols. The drop in Eh value favoured DHA activity at all of tested soil. Our results are similar to that obtained from Luvisol examined by Brzezińska *et al*. (1998). The strongest relationship were observed between DHA activity and pH, in the cases of Terric Histosols and Eutric Fluvisols, whilst the lowest correlation in the case of Mollic Gleysols were stated. Strong relationship between DHA and pH was in agreement with that reported by Qulichano and Marañón (2002) for forest soil.

T a b l e 2. R^2 values with an equations describing the relationship between DHA activity and CO_2 concentration in a headspace, Eh and pH values

Type of soil	Doses of glyphosate ($\mu g\ g^{-1}$)	Relationship between parameters	R^2	Equation
Terric Histosols	0	DHA-CO_2	0.97	$y = 2.4075e^{0.2032x}$
		DHA-Eh	0.72	$y = 81.041e^{-0.0073x}$
		DHA-pH	0.92	$y = 127.66x^2 - 1922x + 7239.5$
	1	DHA-CO_2	0.72	$y = 2.3449e^{0.4534x}$
		DHA-Eh	0.55	$y = 229.22e^{-0.0106x}$
		DHA-pH	0.96	$y = 89.277x^2 - 1343.3x + 5056.6$
	10	DHA-CO_2	0.51	$y = 0.9714e^{0.5629x}$
		DHA-Eh	0.62	$y = 919.1e^{-0.0158x}$
		DHA-pH	0.93	$y = 1E+57x^{-65.215}$
Eutric Fluvisols	0	DHA-CO_2	0.96	$y = 0.9225e^{0.2658x}$
		DHA-Eh	0.88	$y = 2E+08x^{-3.0062}$
		DHA-pH	0.99	$y = 59.761x^2 - 736.32x + 2269.5$
	1	DHA-CO_2	0.93	$y = 0.9056e^{0.2671x}$
		DHA-Eh	0.73	$y = 1E+08x^{-2.9704}$
		DHA-pH	0.99	$y = 56.073x^2 - 704.5x + 2214.3$
	10	DHA-CO_2	0.67	$y = 0.8255e^{0.2889x}$
		DHA-Eh	0.81	$y = 3E+07x^{-2.8167}$
		DHA-pH	0.99	$y = 2.4271x^2 - 15.864x + 4.4608$
Mollic Gleysols	0	DHA-CO2	0.80	$y = 15.741e^{0.4653x}$
		DHA-Eh	0.77	$y = 258.47e^{-0.0076x}$
		DHA-pH	0.61	$y = 1E+17x^{-18.012}$
	1	DHA-CO_2	0.84	$y = 6.2484e^{0.3228x}$
		DHA-Eh	0.87	$y = 161.24e^{-0.0065x}$
		DHA-pH	0.72	$y = 2E+31x^{-34.483}$
	10	DHA-CO_2	0.74	$y = 5.3078e^{0.3141x}$
		DHA-Eh	0.86	$y = 189.32e^{-0.0074x}$
		DHA-pH	0.92	$y = 6E+25x^{-28.246}$

CONCLUSIONS

1. The presence of glyphosate in doses of 1 and 10 μg g^{-1} of soil caused an inhibition of DHA activity in all investigated soils up to 21st day. The 1 μg g^{-1} dose reduced enzymatic activity by 16-20%, while the 10 μg g^{-1} dose reduced the enzymatic activity by 24- 80% in comparison to control samples (without glyphosate supplement).

2. The stronger pesticide effect on DHA activity was found in Terric Histosols, whilst much lower in Eutric Fluvisols.

3. The influence of glyphosate on DHA activity demonstrated significant changes of soil biological conditions even up to 35th day after application.

4. The investigation has shown, that in the case of Terric Histosols and Eutric Fluvisols an increase, but in the case of Mollic Gleysols decrease of carbon dioxide formation took place.

5. Addition of glyphosate to all investigated soils caused an increase of N_2O concentration. Eh, pH and CO_2 concentration had high correlations with DHA activity.

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