J. Elem. s. 129–141

DOI: 10.5601/jelem.2014.19.1.357

CHEMICAL AND ENZYMATIC CHANGES IN SOIL TREATED WITH AMMONIUM GLUFOSINATE*

Stefania Jezierska-Tys, Agata Rutkowska

Chair of Environmental Microbiology
University of Life Sciences in Lublin

Abstract

Intensive use of synthetic chemicals in crop cultivation ensures high crop yields. On the other hand, excessive use of agrichemicals disturbs the biological balance and leads to the contamination of soil environment. The resistance of soil to this type of pollution depends on its physical, chemical and biological properties. Persistence of chemical substances in soil plays a fundamental role in environmental pollution. Therefore, it is important to monitor how long a chemical agent applied to soil remains active. Soil microorganisms and their enzymatic activity are the best indicators of soil contamination with chemical compounds. The aim of this study was to evaluate the influence of the active ingredient (ammonium glufosinate) of the herbicide Basta 150 SL on the microbial activity and chemical properties of soil under winter rape cultivation.

The investigations on the herbicide Basta 150 SL (with ammonium glufosinate as the active ingredient) consisted of a three-year field experiment set up on soil representing the class of black soils. Basta 150 SL was applied to soil in a dose recommended by the producer (2.5 dm^3 ha^{-1}) and at a 10% higher dose (2.75 dm^3 ha^{-1}). Soil was sampled for analyses on 8 dates, i.e. immediately after winter rapeseed harvest and after 2, 10, 12, 14, 22, 24 and 26 months of the experimental period. The study demonstrated that the dose of the chemical agent substantially affected the rate of ammonification and nitrification. A higher dose resulted in the significantly lower rate of the above processes. The manufacturer's recommended dose caused in a significant decrease in the proteolytic activity of soil. None of the applied doses, however, had any significant effect on the soil activity urease. After the application of a recommended dose of Basta 150 SL, the pH_{KCl} values and C:N ratio were higher.

Keywords: soil, enzymatic activity, glufosinate ammonium, Basta 150 SL herbicide.

prof. dr hab. Stefania Jezierska-Tys, Chair of Environmental Microbiology, University of Life Sciences in Lublin, Leszczyńskiego 7, 20-069 Lublin, Poland, e-mail: stefania_tys@op.pl
* This study was supported by grants from the Polish government, the Ministry of Science and Higher Education, under research project No. N N305 410538, in 2010–2013.

ZMIANY CHEMICZNE ORAZ ENZYMATYCZNE GLEBY PODDANEJ DZIAŁANIU GLUFOSYNATU AMONOWEGO

Abstrakt

Intensywne stosowanie syntetycznych substancji chemicznych w uprawach polowych jest niezbędne ze względu na wysokość uzyskiwanych plonów. Nadmierne używanie tych substancji prowadzi do zachwiania równowagi biologicznej oraz zanieczyszczenia środowiska glebowego. Odporność gleby na te zanieczyszczenia zależy od jej właściwości fizycznych, chemicznych i biologicznych. Czas pozostawania substancji chemicznych w glebie ma duże znaczenie w zanieczyszczeniu środowiska naturalnego. Dlatego istotne jest monitorowanie czasu oddziaływania zastosowanego czynnika chemicznego na środowisko glebowe. Mikroorganizmy glebowe i ich aktywność enzymatyczna są najlepszymi wskaźnikami zanieczyszczenia gleby związkami chemicznymi.

Celem pracy była ocena wpływu substancji czynnej (glufosynatu amonowego) wchodzącej w skład herbicydu Basta 150 SL na aktywność mikrobiologiczną oraz chemiczne właściwości gleby pod uprawą rzepaku ozimego.

Badania nad czasem oddziaływania środka chemicznego Basta 150 SL (substancja aktywna – glufosynat amonowy) oparto na trzyletnim doświadczeniu polowym, założonym na glebie należącej do czarnych ziem właściwych. Do gleby wprowadzono środek chemiczny Basta 150 SL w dawce zalecanej przez producenta (2,5 dm^3 ha^{-1}) oraz w dawce zwiększonej o 10% (2,75 dm^3 ha^{-1}). Próbki gleby do analiz pobierano w 8 terminach, tj. bezpośrednio po zbiorze rzepaku ozimego, a następnie po 2, 10, 12, 14, 22, 24 i 26 miesiącach trwania doświadczenia. Wykazano, że na nasilenie procesów amonifikacji i nitryfikacji w glebie istotny wpływ miała dawka badanego środka chemicznego. Zwiększona dawka powodowała istotne osłabienie badanego procesu. Zaaplikowanie do gleby dawki zalecanej przez producenta spowodowało istotny spadek aktywności proteolitycznej. Ponadto stwierdzono, że zastosowane dawki nie wpływały istotnie na aktywność ureazy w badanej glebie. Zaobserwowano również wzrost wartości pH badanej gleby po zastosowaniu preparatu Basta 150 SL w dawce zalecanej oraz stosunku C:N.

Słowa kluczowe: gleba, aktywność enzymatyczna, glufosynat amonowy, herbicyd Basta 150 SL.

INTRODUCTION

Ammonium glufosinate is an active ingredient in the desiccant called Basta 150 SL, popular on winter rapeseed plantations. Ammonium glufosinate (Figure 1), a compound which belongs to aminophosphonates, is an inhibitor of glutamine synthetase and interferes with the photosynthetic process (KIDD, JAMES 1991, PIENIĄŻEK et al. 2003, JARIANI et al. 2010). Consequently, plant tissues become desiccated through dehydration, resulting in severe chlorosis and plant wilting. In soil, ammonium glufosinate is degraded by microorganisms to 3-methylphosphinicopropionic acid (MPP) and 2-methylphosphinicoacetic acid (MPA), and ultimately to carbon dioxide under aerobic conditions (SMITH 1988).

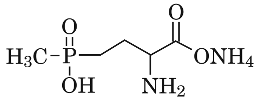

Fig. 1. Formula of ammonium glufosinate

The herbicide Basta 150 SL is a fast acting, contact herbicide, whose mode of action depends on natural biochemical processes occurring in plants. This agent is applied primarily to prepare rapeseed plantations for harvest by accelerating and equalizing plant maturation.

Through an appropriate use of chemical pesticides it is possible to control, either directly or indirectly, their negative impact. However, an inadequate and excessive application of synthetic chemical substances frequently leads to their accumulation in the environment (BEYER, BIZIUK 2007). This may result in the gradual degradation of ecosystems due to the impact of agrichemicals on the biological life of soil and yield quality (SEGHERS et al. 2004; WYSZKOWSKA, KUCHARSKI 2004b). The activity of soil microorganisms and its possible modification due to natural and anthropogenic environmental factors can be assessed via measurements of soil enzymatic activity. The activity of enzymes and biological nitrogen transformations may serve as sensitive indicators of the ecological status of soils (EMMERLING et al. 2002, NANNIPIERI et al. 2003).

The available literature deals with a wide arrays of issues related to the contamination of soil with chemical pesticides, providing data on the detrimental effect of agrichemicals on the microbial abundance and soil enzymatic activity (ROLA, KIELOCH 2001, WYSZKOWSKA 2002*ab*, KUCHARSKI, WYSZKOWSKA 2008). In their research, WYSZKOWSKA and KUCHARSKI (2004*a*) reported inhibitory effects of herbicides on the soil enzyme activity and microbial abundance as well.

The investigations carried out by WIBAWA et al. (2008) demonstrated that ammonium glufosinate applied at doses recommended by the producer did not accumulate in soil. However, PAMPULHA et al. (2007) indicated that application of ammonium glufosinate could stimulate as well as inhibit soil microorganisms, and the nature of specific interactions depended on the concentration of the substance and incubation period. The authors also found that glufosinate ammonium caused inhibition of dehydrogenase activity.

Prolonged and excessive use of synthetic chemical substances in crop cultivation should be looked at with care because of the risk of environmental contamination it creates. Briefly, the aim of the present three-year field experiment was to assess the impact of time- and dose-dependent activity of ammonium glufosinate, an active ingredient of a popular herbicide called Basta 150 SL, on the soil enzymatic activity, i.e. an indicator of soil richness and fertility.

MATERIAL AND METHODS

Characterisation of the soil

A field experiment was set up with the split-block method on soil classified as black soil (WRB-Mollic Gleysols) developed from light silty clay (2-0.5 mm – 65%, 0.05-0.002 mm – 22%, <0.002 mm – 7%). Table 1 presents the basic characteristics of the soil analysed in the study.

Table 1

Basic characteristics of the experimental soil

Parameter	Unit	Value
Reaction	pH_{KCl}	6.1
$C_{org.}$	g kg^{-1} d.m.	9.8
Total nitrogen	g kg^{-1} d.m.	1.3
C:N		7.5
Total phosphorus	g kg^{-1} d.m.	0.7
K	g kg^{-1} d.m.	0.1
Zn	mg kg^{-1} d.m.	34.0
Cd	mg kg^{-1} d.m.	0.15
Cu	mg kg^{-1} d.m.	10.8
Pb	mg kg^{-1} d.m.	9.6
Ni	mg kg^{-1} d.m.	7.5
Cr	mg kg^{-1} d.m.	14.4
Hg	mg kg^{-1} d.m.	0.1
Hh	cmol^{+} kg^{-1} d.m.	1.38

Description of the experiment

An analysis of the impact of Basta 150 SL was based on a three-year field experiment (2010-2012) located at the Experimental Station for Cultivar Testing in Głębokie (52°38'41"N,18°26'18"E). The herbicide Basta 150 SL, containing 150 g of glufosinate ammonium (a compound from the aminophosphonate group) in 1 dm^3 of the agrichemical, was applied in the first year of the experiment. Two doses were tested: recommended by the manufacturer and 10% higher. The experimental model included the following objects:

K – control soil without herbicide;

B1 – soil treated with the optimal (2.5 dm^3 ha^{-1}) dose of Basta 150 SL;

B2 – soil treated with a 10% higher (2.75 dm^3 ha^{-1}) dose of Basta 150 SL.

The herbicide was applied with a knapsack sprayer 8 days before winter rapeseed harvest.

In the first year, seeds of the winter rapeseed cultivar Californium were sown in all objects; sugar beet and rye were cultivated in the second and third year, respectively. During the experiment, basic agrotechnical treatments were conducted and uniform fertilization was applied in accordance with recommendations for the cultivated plants. The surface area of the experimental plots was 12 m^2.

Soil for analyses was sampled from the topsoil layer of each plot eight times, i.e. immediately after winter rapeseed harvest (early August), and next after 2, 10, 12, 14, 22, 24, and 26 months of the experimental period. 20-30 soil samples were collected from each experimental object; after mixing, they formed a 6-kg composite sample.

Biochemical and chemical analyses

The soil was transported in heat-proof polyethylene bags at a low temperature. In a laboratory, the soil material was thoroughly sieved through 2-mm mesh. Soil from averaged samples was analysed and used for determination of protease activity with the method of LADD and BUTLER (1972) modified by ALEF and NANNIPIERI (1995), in which sodium caseinate served as a substrate; the activity of urease was assayed with the method of ZANTUA and BREMNER (1975) modified by ALEF and NANNIPIERI (1995), with urea as a substrate; ammonification rate was assessed with the Nessler's method while nitrification was analyzed with the brucine method (NOWOSIELSKI 1981). Chemical analyses included determination of the content of organic C by the Tiurin method; total N by the Kjeldahl method; total P spectrophotometrically; K by atomic emission spectrometry; Zn, Cd, Cu, Pb, Ni, Cr, Hg by atomic absorption spectrometry. The soil pH in 1M KCl was determined potentiometrically.

Statistical analyses

The results were analysed statistically with analysis of variance. The least significant differences were calculated with the Tukey's test at the significance level α = 0.05. All statistical calculations were performed using Statistica 7.1 software.

RESULTS AND DISCUSSION

Intensity of ammonification and nitrification processes

Nitrogen is one of the major elements in the earth and its soil transformations are related to the level of C, which provides energy for microorganisms involved in these processes (EMMERLING et al. 2000). The content of nitrogen in soil is closely linked with organic matter (SCHNITZER 2001).

Figure 2 presents the effect of the herbicide Basta 150 SL applied during the field experiment on increasing the rate of ammonification in soil under rapeseed. The present study has shown considerable fluctuations in the process of ammonification in soil. The analysis of the results implies that initially the level of ammonification in soil treated with the recommended dose of the herbicide was significantly lower than in the control object. In turn, the 10% higher dose reduced ammonification to zero. Later, on the second sampling date, the rate of ammonification upon the application of the optimal herbicide dose declined substantially in comparison with the control object. Following an application of Basta 150 SL in a dose higher than recommended by the producer, the rate of ammonification was similar to the control. On the subsequent dates, periodic fluctuations in the intensity of ammonification were observed. Worth noticing was a significant increase

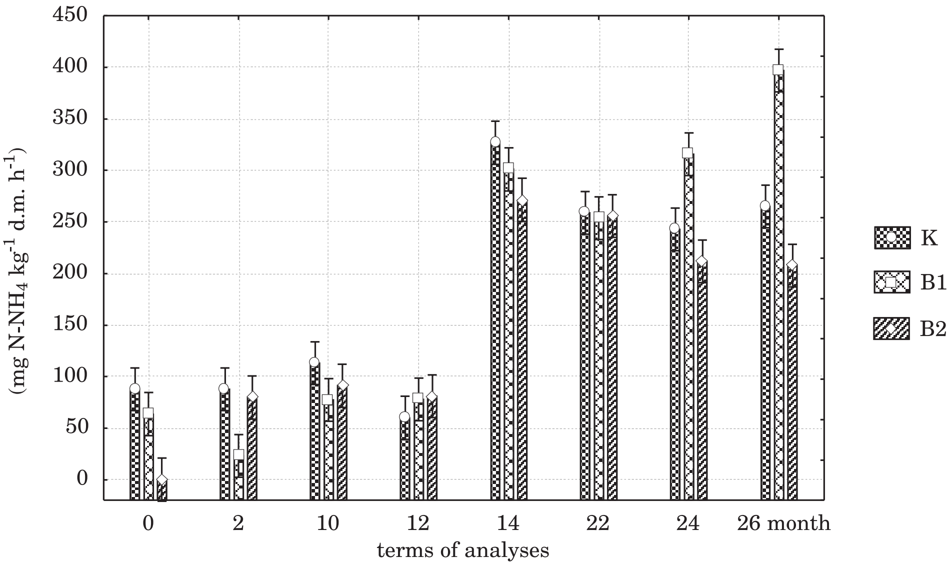

Fig. 2. Periodic intensification of the ammonification process in individual experimental objects: K – control soil without herbicide addition, B1 – soil treated with the optimal dose of Basta 150 SL herbicide, B2 – soil treated with the 10% higher dose of Basta 150 SL herbicide. Vertical bars indicate the 0.95 confidence interval

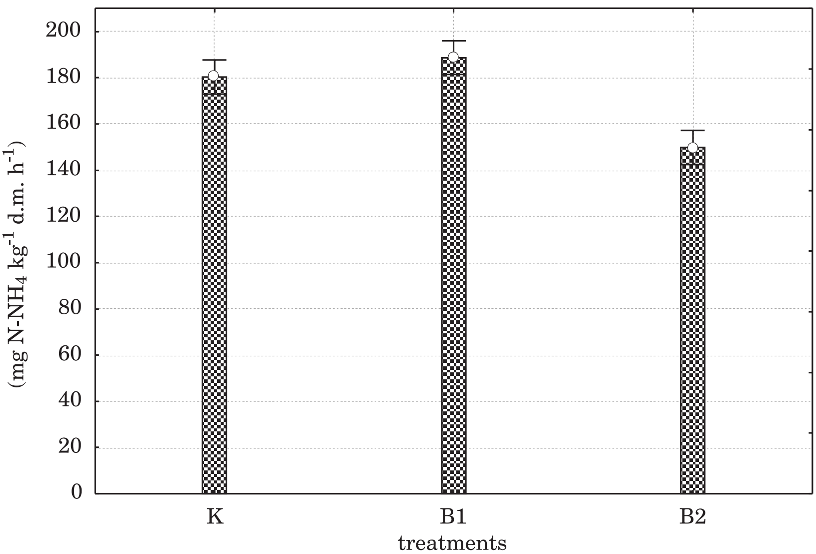

Fig. 3. Mean values of ammonification in individual experimental objects
Key: cf. Fig. 1

in the rate of ammonification 24 and 26 months after the application of the chemical agent in the manufacturer's recommended dose. Our analysis of the mean values from the entire experimental period and experimental objects (Figure 3) demonstrated a significant decrease in ammonification induced by Basta 150 SL applied in the higher dose; this may have been related to the $-NH_2$ group, which is a constituent of ammonium glufosinate. Investigations conducted by other authors (STRZELEC 1986, KRZYŚKO-ŁUPICKA 2008) revealed a stimulating effect of Roundup (containing glyphosate as an active ingredient) on the enhancement of ammonification in soil, which may indicate that the preparation is used up by microorganisms. KUCHARSKI et al. (2009) investigated the effect of a variety of herbicides (Harpun 500 S.C., Fawory 300 SL, Akord 180 OF, Mocarz 75 WG) on the course of ammonification in soil. They reported that Mocarz 75 WG caused the highest inhibition of ammonification in tested soil. Additionally, the authors showed that the type of herbicide, dosage and experiment's duration influenced the course of the process.

Figure 4 presents the results of determinations of the $N-NO_3$ content in the experimental objects. Comparing these results, it appears that changes in the rate of nitrification induced by the application of two different doses of Basta 150 SL were subject to periodic fluctuations. On the first sampling date, as well as 2, 22, and 24 months after the treatment, Basta 150 SL applied in the manufacturer's recommended dose significantly intensified nitrification versus the control object. In turn, the 10% higher dose significantly inhibited the process in 2nd, 10th, 12th and 26th month. The data presented in Figure 5 indicate significant stimulation by the recommended dose throughout the study period, and significant inhibition induced by the elevated

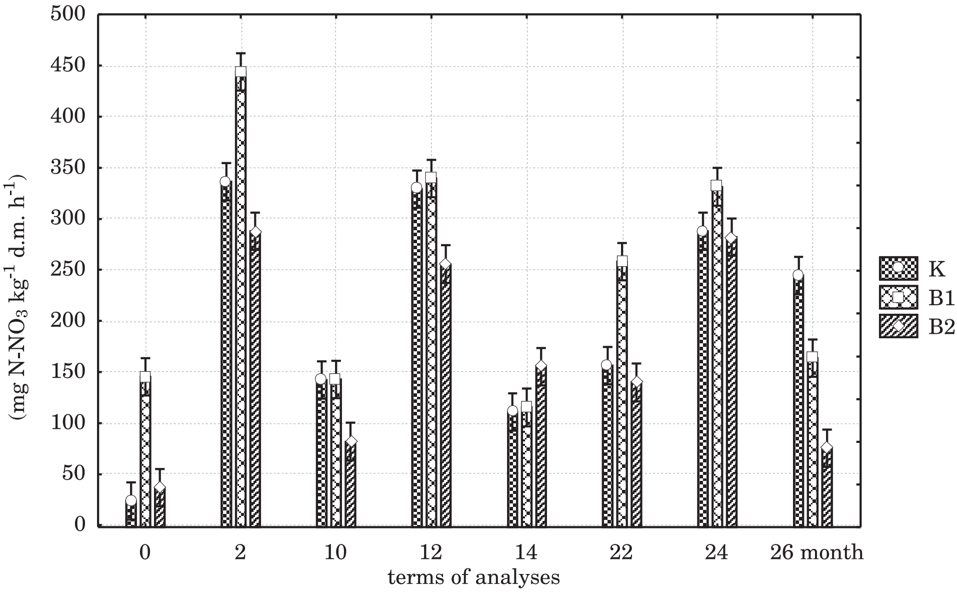

Fig. 4. Periodic intensification of nitrification in individual experimental objects
Key: cf. Fig. 1

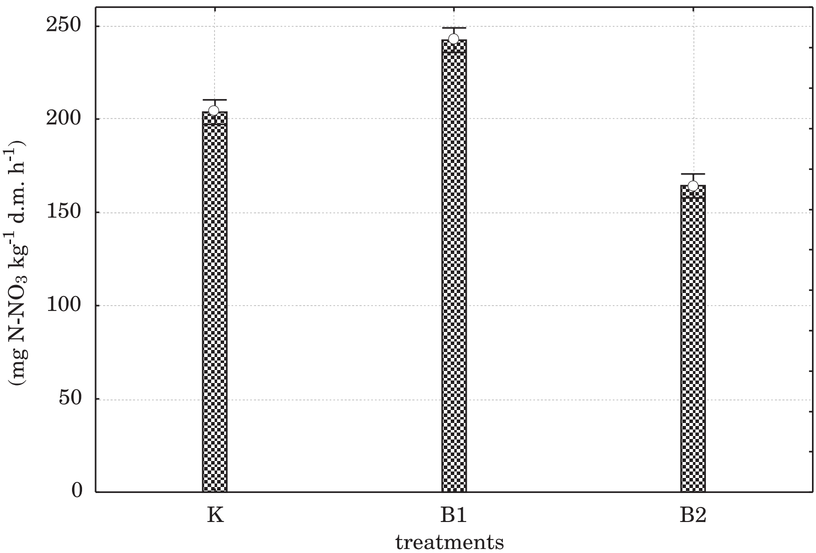

Fig. 5. Mean values of nitrification in individual experimental objects
Key: cf. Fig. 1

dose. Similarly, in their investigations on application of optimal doses of chemicals, Swoboda et al. (2007) reported an increased content of nitrate nitrogen compared with control objects.

Activity of protease and urease

The activity of protease measured with the hydrolytic activity towards casein represents the activity of extracellular enzymes (Ladd and Butler 1972). Figure 6 shows the activity of the enzyme in response to the two doses of Basta 150 SL. Changes in the activity of protease, which plays an essential role in mineralization of organic nitrogen, were found in individual soil objects. Based on the results, it was concluded the above process was stimulated in the first time period. A significant decline in the proteolytic activity was reported 2, 10, and 12 months after the application of the recommended dose, which had a considerable impact on the mean value from this object (Figure 7). The decrease in the activity was significant against both the control and the object treated with higher dose.

The activity of urease in soil treated with the different doses of the herbicide Basta 150 SL is illustrated in Figures 8 and 9. The data indicate that the two herbicide doses significantly increased the enzyme's activity compared to the control in the first study period. In contrast, 2 and 26 months after the application, both the optimal dose and 10% higher doses caused a significant decline in the ureolytic activity in soil. Throughout the experiment, periodic fluctuations in the enzyme's activity were observed. The mean values obtained during the entire experiment corresponded to the va-

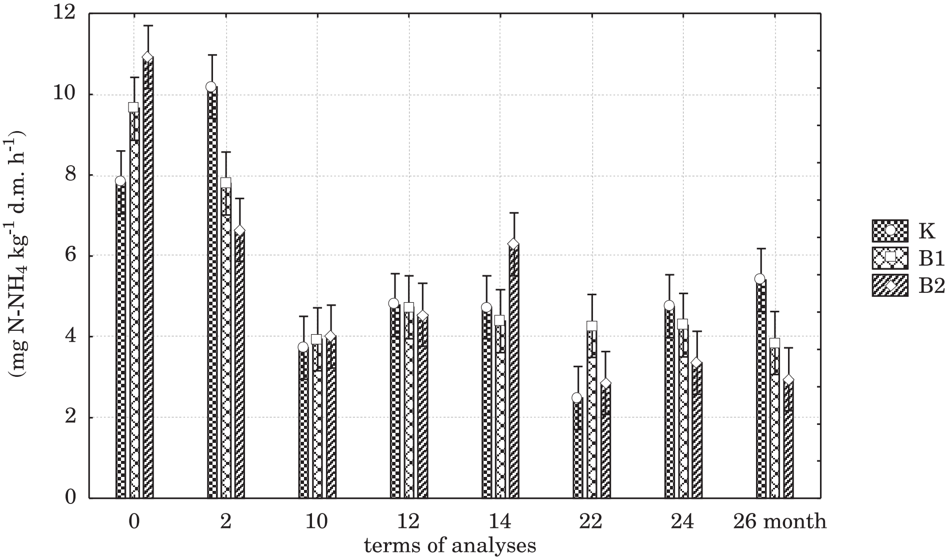

Fig. 6. Periodic activity of urease in individual experimental objects
Key: cf. Fig. 1

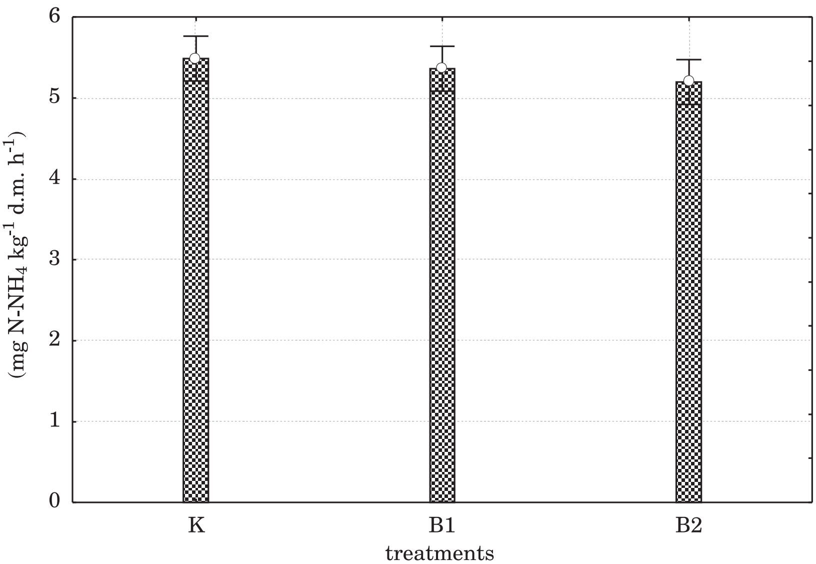

Fig. 7. Mean urease activity in individual experimental objects
Key: cf. Fig. 1

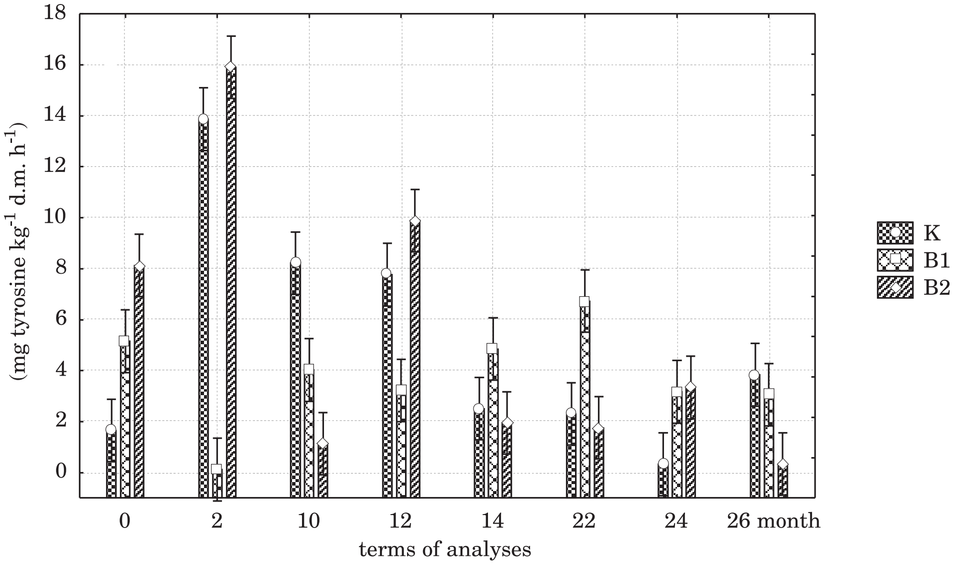

Fig. 8. Periodic activity of protease in individual experimental objects
Key: cf. Fig. 1

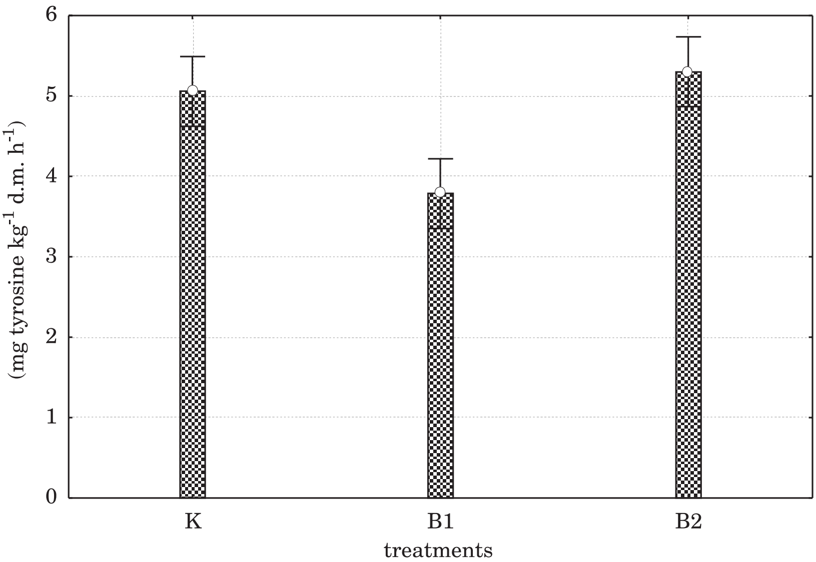

Fig. 9. Mean protease activity in individual experimental objects
Key: cf. Fig. 1

lues obtained in the control soil. Therefore, it can be concluded that neither the recommended nor the increased dose triggered significant changes in the activity of urease. Similar results were obtained by WYSZKOWSKA (2002a) and KUCHARSKI and WYSZKOWSKA (2008) and BAĆMAGA et al. (2012), who found a negative effect of herbicides on urease in soil.

pH_{KCl} of soil

The results concerning pH are presented in Table 2. In general, throughout the experiment, Basta 150 SL did not have any significant effect on the pH value of soil, although a periodic decline or rise in the reaction was observed. It is noteworthy that the pH value increased immediately after application of the chemical agent at both the recommended and 10% higher dose.

Table 2

Periodic soil pH_{KCl} values in individual experimental objects during the experiment

Experimental objects	Analysis period month							
	0	2	10	12	14	22	24	26
Control soil without herbicide addition (K)	5.6	7.1	6.1	7.0	6.8	6.6	6.6	7.1
Soil treated with the optimal dose of Basta 150 SL herbicide (B1)	6.5	7.0	6.2	7.0	6.7	6.5	6.5	7.1
Soil treated with the 10% higher dose of Basta 150 SL herbicide (B2)	6.8	7.1	6.8	6.9	7.1	6.8	6.8	6.7

Content of organic carbon, total nitrogen, and C:N

Our analysis of the mean organic carbon content (Table 3) showed that C_{org} increased in response to the application of the recommended dose of Basta 150 SL (11.4 g kg^{-1} d.m.). A similar tendency was found for the nitrogen content. The highest N_{org} content (1.4 g kg^{-1} d.m.) was found in the soil treated with the recommended dose of Basta 150 SL. The lowest value of the C:N ratio was found in the experimental objects in 2010, when it reached 8.2 in the control soil and 8.8 in the soil treated with the increased Basta 150 SL dose. Furthermore, with time, the C:N ratio was found to have risen by 27% in the soil treated with the higher dose of the herbicide Basta 150 SL.

Table 3

Content of organic carbon, total nitrogen, and C:N in the individual experimental objects during the experiment

Experimental objects	Year	Parameter		C:N
		C_{org} (g kg^{-1} d.m.)	total N (g kg^{-1} d.m.)	
Control soil without herbicide addition (C)	2010	9.8	1.3	7.5
	2011	10.8	1.2	9.0
	2012	11.6	1.3	9.0
Mean		10.7	1.3	8.2
Soil treated with the optimal dose of Basta 150 SL herbicide (B1)	2010	10.4	1.4	7.4
	2011	11.8	1.3	9.1
	2012	12.0	1.4	8.6
Mean		11.4	1.4	8.1
Soil treated with the 10% higher dose of Basta150 SL herbicide (B2)	2010	9.4	1.2	7.8
	2011	10.5	1.1	9.5
	2012	11.9	1.2	9.9
Mean		10.6	1.2	8.8

CONCLUSIONS

1. The dose of the herbicide applied had a significant effect on the rate of ammonification in soil.
2. The proteolytic activity was significantly the highest after the application of Basta 150 SL in the dose recommended by the producer.
3. The applied doses did not significantly affect the activity urease in soil.
4. The herbicide Basta 150 SL used in the recommended and increased doses caused an increase in the pH reaction of soil and in the C:N ratio.

REFERENCES

Alef K., Nannipieri P. 1995. *Protease activity.* In: *Methods in applied soil microbiology and biochemistry.* Eds K. Alef and P. Nannipieri. Academic Press. London, pp. 576.

Baćmaga M., Boros E., Kucharski J., Wyszkowska J. 2012. *Enzymatic activity in soil contaminated with the Aurora 40 WG herbicide.* Environ. Protect. Eng., 38(1): 91-102.

Beyer A., Biziuk M. 2007. *Methods of determination of residues of pesticides and polychlorinated biphenyls in food samples – a review.* Ecol. Chem. Eng., 14, S3: 35- 58.

Emmerling C., Liebner C., Haubold-Rosar M., Katzur J., Schröder D. 2000. *Impact of application of organic waste materials on microbial and enzyme activities of mine soils in the Lusatian coal mining region.* Plant Soil, 220: 129-138.

Emmerling C., Schloter M., Hartmann A., Kandeler E. 2002. *Functional diversity of soil organisms – a review of recent research activities in Germany.* J. Plant Nutr. Soil Sci., 165: 408-420.

Jariani S.M.J., Rosenani A.B., Samsuri A.W., Shukor A.J. Ainie H.K. 2010. *Adsorption and desorption of glufosinate ammonium in soils cultivated with oil palm in malaysia*. Malays. J. Soil Sci., 14: 41-52.

KIDD H, JAMES DR. 1991. *The agrochemicals handbook*. Third Edition. Royal Society of Chemistry, Information Services, Cambridge, UK, pp. 3-11.

KRZYŚKO-ŁUPICKA T. 2008. *Ecological effects of phosphoorganic herbicide on soil diazotrophs in spring.* Part II. Ecol. Chem. Eng., 15(4): 596-602.

KUCHARSKI J., BAĆMAGA M., WYSZKOWSKA J. 2009. *Effect of herbicides on the course of ammonification in soil.* J. Elementol., 14(3): 477-487.

KUCHARSKI J., WYSZKOWSKA J. 2008. *Biological properties of soil contaminated with the herbicide Apyros 75 WG*. J. Elementol., 13(3): 357-371.

LADD J.N., BUTLER J.H.A. 1972. *Short-terms assays of soil proteolytic enzyme activities using proteins and dipetide derivatives as substrates.* Soil Biol. Biochem., 4: 19-30.

NANNIPIERI P., ASCHER J., CECCHERINI M.T., LANDI L., PIETRAMELLARA G., RENELLA G. 2003. *Microbial diversity and soil functions*. Eur. J. Soil Sci., 54: 655-670.

NOWOSIELSKI O. 1981. *Methods for determination of fertilization demand*. PWRiL, Warszawa. (in Polish)

PAMPULHA M.E., FERREIRA M.A., OLIVEIRA A. 2007. *Effects of a phosphinothricin based herbicide on selected groups of soil microorganisms.* J. Basic Microbiol., 47(4): 325-31.

PIENIĄŻEK D., BUKOWSKA B., DUDA W. 2003. *Glyphosate - a non-toxic pesticide?.* Med. Pracy, 54 (6): 579-583. (in Polish)

ROLA H., KIELOCH R. 2001. *Effect of herbicides on yield, health and chemical composition of seeds of selected yellow and white lupie cultivars*. Biul. Nauk. UWM, 12: 47-55. (in Polish)

SCHNITZER M. 2001. *Nitrogen chemistry in soils.* In: *Chemistry for XXI century.* KEINAN E. & SCHECHTER I. (eds). Wiley-VCH GmbH, Weinheim, 117-129.

SEGHERS D., WITTEBOLLEV L., TOP E. M., VERSTRAETE W., SICILIANO S. D. 2004. *Impact of agricultural practices on the Zea mays L. endophytic community.* Appl. Environ. Microbiol., 70(3): 1475-1482.

SMITH A.E. 1988. *Persistence and transformation of the herbicide [14C] glufosinate-ammonium in prairie soils under laboratory conditions.* J. Agric. Food Chem., 36(2): 393-397.

STRZELEC A. 1986. *Effect of soil properties on response of soil microflora to herbicides.* Rocz. Glebozn., 37 (1): 129. (in Polish)

SWOBODA T., CYCOŃ M., KACZYŃSKA A. 2007. *The respiratory and nitrifying activity as basic parameters for evaluation of the influence of herbicides on microorganisms involved in carbon and nitrogen cycling.* Ekotoksykologia w Ochronie Środowiska Glebowego i Wodnego. IUNG-PIB Puławy. (in Polish)

WIBAWA W., ROSLI BIN MOHAMAD R., AGAM BIN PUTEH A., OMAR D., JURAIMI A.A., ABDULLAH S.A. 2009. *Residual phytotoxicity effects of paraquat, glyphosate and glufosinate-ammonium herbicides in soils from field-treated plots.* Int. J. Agric. Biol., 11(2): 214-216.

WYSZKOWSKA J. 2002*a*. *Effect of soil contamination with Trefla 480 EC on biochemical properties of soil*. Pol. J. Environ. Stud., 11(1): 71-77.

WYSZKOWSKA J. 2002*b*. *Number of cellulolytic, ammonifying, nitrogen immobilizing and Azotobacter sp. bacteria in soil contaminated with Treflan 480 EC*. Pol. J. Ntur. Sci., 10(1):71-83.

WYSZKOWSKA J., KUCHARSKI J. 2004*a*. *Biochemical and physicochemical properties of soil contaminated with herbicide Triflurotox 250 EC.* Pol. J. Environ. Stud., 13(2): 223-231.

WYSZKOWSKA J., KUCHARSKI J. 2004*b*. *Biological properties of soil polluted with Chwastox Trio 540 Sl.* Rocz. Glebozn., 50: 311-319. (in Polish)

ZANTUA M.J., BREMNER J.M. 1975. *Comparison of methods of assaying urease activity in soils.* Soil Biol. Biochem., 7: 291-295.