INFLUENCE OF XENOBIOTICS ON THE BIOLOGICAL SOIL ACTIVITY
VPLYV XENOBIOTÍK NA BIOLOGICKÚ AKTIVITU PÔDY

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ABSTRACT

The both basic and potential biological soil activity along with a biological degradation capacity of soil microorganisms through their respiratory activity were investigated after the application of polymers with the different degree of degradation in the soil. The CO_2 production was measured by the absorption method at standard moisture and temperature under laboratory conditions. Numerous representing changes of major soil microbial groups were determined after the application of the polyethylene. Polyethylene (PE), polyvinylalcohol (PVA), polyvinylalcohol modified by hydrolysed collagen (PVAHC) were applied as synthetic polymers into luvisol. The addition of all tested polymers significantly influenced the CO_2 production. During 43 days of incubation period, the total amount of produced CO_2 was 1271.6 $mg.kg^{-1}$. The total amount of mineralised carbon in the soil reached 4.55 %. When compared with the soil, the respiratory activity of soil microorganisms increased 3-times, 1.2-times and 1.3-times after addition of starch, PVA and PVAHC, respectively. The addition of granular PE reduced the soil pores size, which resulted in a significant decrease of CO_2 production and non-significant rearrangement of the major microbial groups in the soil after three month of incubation.

KEY WORDS: respiration of soil microorganisms, the biological degradation, polyethylene, polyvinylalcohol

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INTRODUCTION

Organic carbon is an essential nutrient source for most soil microorganisms, but not all the organic substances and their forms are utilisable by microflora. Mainly, the group of recalcitrant substances, which include synthetic polymers, is becoming to be problematical for a soil, as well as for an environment since synthetic polymers are either decomposed for very long time period or are not decomposed in the soil [5, 3].

These substances are able to persist at steady state in the soil and cause significant ecological problems. During their decomposition, undesirable degradation products can be formed that are able to subvert natural biological soil activity and, thus cause the changes of CO_2 production. The degradation of organic substances is mostly evaluated through the determination of microbial growth, chemical and physical changes, O_2 consumption or CO_2 production [3]. The supply of mineral nitrogen forms, increased concentration of the xenobiotics as well as other pollute substances along with moisture, temperature and intake of O_2 belong to most important factors affecting respiratory activity of soil micro-organisms. Heavy metals, petroleum, pesticides, herbicides and fertilisers are frequently monitored substances in soils. Soils that were either fertilised by mineral nitrogen or polluted by high doses of heavy metals, such as cadmium at concentration level higher than 400 $mg.kg^{-1}$, have decreased of respiratory activity of soil microflora [11]. However, [7] reported that soil respiratory activity decreased at much lower doses of heavy metals, i.e. cadmium at the concentration ranging from 0.15 to 0.66 $mg.kg^{-1}$. On the other hand, mentioned authors also observed that soil respiratory activity increased after petroleum pollution and contamination of soil by magnesium ranged from 1.54 to 2.18 $g.kg^{-1}$.

There is a lack of information about the fate of other xenobiotics persisting in arable soil. In addition, the determination of respiratory activity itself is only aimed to detect and calculate the decomposition degree of tested xenobiotic. These studies are mostly carried out in the activated sewage-sludge.

In consequence of the above mentioned problems, our study was aimed at monitoring of alternations of the biological soil activity in the soils reached by polymer compounds with the different degradation ability. From hardly decomposable polymers, polyethylen was applied into the row soil. This recalcitrant substance has been found to be decomposed only by 0.26 ± 0.04 % from the original weight under aerobic laboratory conditions during one year period, using the mixed culture of the microscopic fungi [1]. Considering polyethylene foils, the decomposition of these substances takes relatively long time under natural conditions and as a rule not even ten years lasting period does not seem to be sufficient for the significant increase of biodegradation range or for their total mineralisation [2]. In agriculture, PE is applied into soil in particular during the soil surface covering in the form of foils, which causes problems with its own decomposition after the termination of vegetation period. One of the possibilities how to solve these problems is to manufacture biodegradable PE foils which contain highly decomposable substance, such as starch. Regarding the environment, however, manufacturing and application of the total biodegradable mulching-foils based on β-hydroxybutyrate and β-hydroxyvalerate would be acceptable as the best approach [8]. Using such kind of foils, there would not remain any visible scraps and the foils would be fully decomposed after termination of vegetation period.

The next compound - PVA is usually applied into the soil as a polymer carrier for pesticides, herbicides, and seed treatment product. Among all vinylpolymers, PVA is an easy biodegradable polymer substance. The reason is that majority of vinylpolymers can not be hydrolysed. PVA behaves as a recalcitrant in dry state only, but in a water solution is soluble and becomes biodegradable. Both the solubility and degree of PVA degradation increase with decreasing the number of acetate groups in PVA molecule. In order to increase the biodegradation of PVA foils (similarly as in PE ones), it seems to be effective to incorporate easier decomposable additives into their basic chemical chain from the group of natural polysacharides, such as starch or cellulose [9]. Microbial degradation of PVA is frequently described as an enzyme degradation of alcohol groups by peroxidases isolated from the soil bacteria, such as *Pseudomonas sp*. The decomposition is initiated by enzyme oxidation of the secondary alcohol group on the

ketonic group. Following the hydrolysis of ketonic group it is resulted in chain breaking [6]. Partial degradation of PVA was also confirmed by measuring respirometry to BSK_{60} (O.22 $\pm$ 0.04) in aerobic reactor, whereas in PE, the BSK_{60} was measured only 0.006 under the same conditions [8].

MATERIAL AND METHODS

50 g of fluvisol for each experimental vial was used in laboratory model experiment. The soil was collected at the location of Research and experimental base in Malanta, Slovak agricultural university. 100 mg of tested polymer substances were added into the soil. The soil was wattered upto 60% WHC (water holding capacity) in all the samples. The samples of PVA and starch were applied in the soluble form. The sample of PE was applied into the soil in the insoluble state as a granulate. Unamemdent soil was considered as a control treatment. 1 ml volume of soil solution, prepared from the biologically active soil with a titter of $0.82.10^6.ml^{-1}$, was used to inoculate each sample. The samples were kept at +25 ^oC during whole incubation period.

The fluvisol was used with the following characteristics such as: 1.27% C_{ox}; 0.149% N_t; pH H_2O: 6.85; pH KCl: 5.57; C:N = 8.5:1; soil moisture: 13 %; $N\text{-}NH_4^+$: 0.35 $mg.kg^{-1}$ dry soil; $N\text{-}NO_3^-$: 3.50 $mg.kg^{-1}$ dry soil; biomass of microorganisms (fumigation method): 180 μg $C.g^{-1}$ dried soil.

The changes of the respiratory activity of the soil micro-organisms were investigated after the addition of four polymer substances. These polymer substances (polymers) have usually been applied into the soil during different agricultural activities. Besides of the basic components, polyvinylalcohol (PVA) contained also glycerol, stearine, and SiO_2. This polymer is produced by Slovak company SLOVIOL, Chemical Plant Nováky, with hydrolysis of 88 mol %. The polymer was applied as a plastic material, containing 52.86 wt% of carbon (calculation without stearine).

Polyvinylalcohol in the plastic form with the addition of hydrolysed collagen (PVAHC) was also applied as a modification of the previous plastic material. The carbon content of this material was 49.85%. It is produced by Department of Plastic Materials and Rubber (CHTF STU Bratislava, Slovakia). Added hydrolysed collagen with average molecular weight of 2000, nitrogen content of 14.7 wt%, and viscosity of 4% wt.water solution at 20 ^0C was produced by KORTAN Company.

Among easy decomposable polymer substances, the chemically pure and water soluble starch with carbon content of 44.44 wt% was used in the experiment. From only hardly decomposable polymer substances, polyethylene (PE) with a trade name Bralen FB2-17 LDPE (low density polyethylene) was also used. This polymer is insoluble in water and resistant to non-oxidising acids, hydroxides, salts, and their solutions. The substance was produced by SLOVNAFT Company (Bratislava, Slovakia) and applied in the granulate form, but not in a plastic form, in order to achieve increased surface contact between the polymer and soil. The carbon content of 85.71 wt% was used to do correct calculations.

Both the biological soil activity as well as the biodegradation potential of the soil microflora were investigated in soil medium after addition of tested polymers. Respiratory activity measurement was carried out as follows: CO_2 was retained into 0,1N KOH solution and the amount of absorbed CO_2 was determined by titration with 0,1N HCl solution after followed addition of saturated $BaCl_2$ solution [10, 4, 12]. Production of CO_2 was measured within 43 days, each treatment in five replicates. CO_2 assessments timing was carried out according to intensity of respiration as follows: 7-times after 24 hours, 4-times after 48 hours, 4-times after a week (7 days).

The changes in quantity of major microbial groups were determined only in two treatments, in the first one with the unamended soil and in that one with the soil amended with polyethylene. This analysis was carried out after finishing the 3-month (92 days) lasting cultivation period. Population densities of following microorganisms were determined: bacteria and their spores utilising organic nitrogen on the meat-peptone agar, bacteria and their spores utilising inorganic nitrogen on the Thorton's agar (TA) and microscopic fungi were determined using both the Czapek-Dox's agar (Cz-DA) and malt´s extract agar (MA). The plate-diluting method was used to determinate quantity of the individual physiological groups of microorganisms in the soil, each in three replicates. Microbial determination was performed prior to test establishment, results are presented as

control (C), and at the end of this experiment – on the 92nd day of incubation.

Determined data were statistically evaluated by Kruskal-Wallis´s test, using computer programme STATGRAPHICS (Stehlíková unpublished).

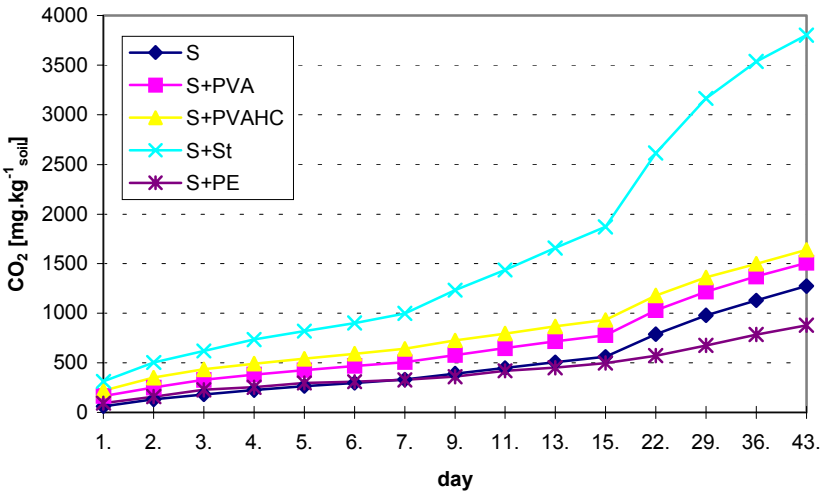
RESULTS AND DISCUSSION

CO_2 emitted during decomposition of the organic substances is considered to be the most complex parameter of mineralization of organic substances in the soil [11] as well as the indicator of biological degradation of organic substances ASTM 5209.

CO_2 production during incubations of the soil samples is shown in Figure 1. The adaptation phase of the microorgnisms (lag-phase) was short, especially in the treatments with the addition of starch (S+St:soil with starch) and polyvinylalcohol (S+PVA : soil with PVA). Present soil microflora was able to adapt to organic substrates immediately after their addition. When compared with starch treatment (S+St), where the highest amount of CO_2 evolved was determined after 24 hours, there was observed also a positive effect of addition of all the polymer substances tested on microbial respiration. In the unamended soil (S), the maximum amount of respirated CO_2 was observed 24 hours later when compared with treatments containing starch and polyvinylalcohol (S+PVA). The determined values of CO_2 evolved confirmed that the unamended soil sample had also very good biological activity with sufficient supply of organic carbon and nitrogen (see methods).

In all evaluated samples, the highest amount of respiratory CO_2 was determined during the first week of incubation period. In the samples amended with the S+PVAHC (soil with PVAHC), polyethylene (S+PE), S+PVA, and row soil (S), 39%, 35.1 %, 33.6 %, and 26 % of respiratory CO_2 were determined, respectively. In all treatments, the half of respired CO_2 was retained during the first two weeks of decomposition of organic substances in the soil. During the following weeks, CO_2 production had decreasing tendency. The same decreasing tendency was only observed in soil treatment by starch. In other soil treatments, a strong decline in CO_2 production was found after one week of lasting incubation period. Addition of polymer substances, except of polyethylene, had a positive effect on the soil respiration activity.

Figure 1.: The course of CO_2 production (cumulative values) in luvisol



The obtained data of CO_2 production were statistically analysed (Table 1-3). Based on the obtained basic statistical variation characteristics (Table 1), the file of measured CO_2 values can not be considered as balanced one, since the value of variation coefficient was higher than 50%.

Table 1.: The basic variance-statistical characteristics and results from distribution fitting the CO_2 production and population density of soil microorganisms

Statistical characteristics	CO_2 [$mg.kg^{-1}_{soil}$]					Number of microorganisms [$KTJ.g^{-1}_{soil}$]	
	Variants					Variants	
	S	S+PVA	S+PVAHC	S+St	S+PE	S	S+PE
n	75.00	75.00	75.00	75.00	75.00	18.00	18.00
x	4.24	5.02	5.33	12.62	2.93	$6.38x10^{6}$	$4.63x10^{6}$
s	3.07	3.13	3.14	8.88	1.59	$9.72x10^{6}$	$6.41x10^{6}$
s^2	9.45	9.81	9.83	78.89	2.53	$9.46x10^{13}$	$4.11x10^{13}$
v [%]	72.55	62.35	58.84	70.38	54.36	154.41	138.34
Distribution fitting							
χ^2	97.30	54.59	51.97	21.34	8.07	4.78*	
P-value	0.00	$5.30x10^{-9}$	$1.71.10^{-8}$	$1.59.10^{-3}$	0.33	$4.16.10^{-6}$*	

n -count, x - average; s - standard deviation; v - coefficient of variation; χ^2 - chi square test; P-value- significant level; S-soil; St-starch; PVA - polyvinylalcohol; PVAHC-polyvinylalcohol with hydrolyzed collagen; PE-polyethylene, *data are calculated from the whole file (S and S+PE) because numbers of degree of free was insufficient

For this reason, Kruskal-Wallis' test was selected for next statistical data processing. In accordance with Kruskal-Wallis' test (Table 2), the significant differences were found between both the individual treatments as well as days of the CO_2 production measurements. Considering the biological aspect of the topic, significant differences can be assumed as a result of uneven intervals between measurements, which have met our expectations. Considering individuals treatments, high significant effect on CO_2 production was determined only in the treatments which were amended with PVAHC and starch as compared with the soil.

High significant differences were registered between the following treatments: S to S+St, S+PVA to S+St and S+PE, S+ PVAHC to S+St and S+PE, S + St to S+PE (Table 3).

Table 2.: Analysis of variance according to Kruskal-Wallis test with replicates in time for production CO_2 (n=375) and determined group of microorganisms (n=36)

Source of variability	Count	d.f.	Test statistic	Significant level
Production of CO_2 (n=375)				
Treatments	75	4	130.94	0.00 ++
Measurements timing	25	14	202.00	0.00 ++
Replicates	75	4	0.19	0.99
Determined group of microorganisms (n=36)				
Treatments	18	1	0,82	0.37
Physiological group of microorganisms	6	5	25,51	$1,11.10^{-4}$ ++
Replicates	3	2	0,73	0.69

Table 3.: Contrast estimation for treatments (CO_2) and physiological group of microorganisms according to Dunn for α=0,01

Source of variability	Count	Average rank	Contrast
Treatments			
S	75	143.96	A
S+PVA	75	180.93	B C
S+PVAHC	75	198.91	D E
S+St	75	301.90	A B D F
S+PE	75	114.30	C E F
Physiological group of microorganisms			
B→ MPA	6	28.33	A
SB→ MPA	6	25.75	
B→ TA	6	23.92	
SB→ TA	6	20.00	
MF→Cz-DA	6	7.08	
MF→MA	6	5.92	A

$z(\alpha,n,k)$ - tabulated value; $z_{(0,05,\ 5,\ 75)} = 49.74$; $z_{(0,01,\ 5,\ 75)} = 61.95$; $z_{(0,05,\ 6,\ 6)} = 17,82$; $z_{(0,01,\ 6,\ 6)} = 21,29$;
A, B, C, D, E,F - contrasts

Biodegradation processes in all treatments are introduced in the table 4 that includes values of respiratory CO_2 after 43 days of lasting cultivation period. Calculations were made according to data of tested polymers provided by their producer as well as theoretical knowledge about respiration activity of the soil microorganisms. The amount of soil carbon was calculated according to determined content of C_{ox}. When the totally mineralised carbon was calculated, both the respiratory carbon as well as carbon immobilised in microbial bodies were taken into consideration. According to [10], the amount of respiratory carbon from the total amount is 60 %, while immobilised carbon takes only 40 %. According to our calculation, we have found that 4.55 % of carbon was mineralised under laboratory conditions during 43 days. The addition of the polymers increased the activity of micro-organisms respiration by 8.17 % in starch treatment. The addition of PVA and PVAHC increased the amount of the totally mineralised carbon by to 0.43% and 0.89%, respectively. The application of PE, however, caused reduction of mineralisation of microbial activity by 1.78% in the soil. Thus, it can be assumed that polyetylene inhibited respiration activity of the soil micro-organisms.

Table 4.: Microbiologically mineralised carbon in the samples after 43 days incubation period

Treatments	Respiratory CO_2 after 43 days	Carbon in sample	Respiratory carbon	Immobilised carbon	Mineralised carbon	Mineralised carbon in sample
	[$mg.kg^{-1}_{soil}$]					[%]
S	1271.60	12700.00	346.76	231.17	577.93	4.55
S+PVA	1507.40	13757.20	411.07	274.05	685.12	4.98
S+PVAHC	1638.60	13697.00	446.85	297.90	744.75	5.44
S+St	3802.40	13588.80	1036.91	691.27	1728.18	12.72
S+PE	878.40	14414.20	239.54	159.69	399.23	2.77

High level of soil respiration can be caused either by high activity of the small community of soil micro-organisms or by low activity of the prevalent community of micro-organisms [11]. In fact, this

parameter can not reliably indicate neither initial soil processes changes nor toxic influence of xenobiotics on the soil microbial communities. This lack of respirometry determination tests was eliminated by determination of changes of population densities within physiologically important microbial groups in the soil prior to incubation period (Table 5).

Table 5.: Effect of powder polyethylene on soil microbial communities before experiment establishment (treatment C) and at the end of experiment (treatments S and S+PE) after 93 days of incubation period.

Observation group of Micro-organisms	Microbial agar	Treatments		
		C	S	S+PE
Bacteria and spores [10^6 CFU. ml^{-1}]	B→ MPA	2.64	23.03	16.62
	SB→ MPA	2.34	7.17	4.16
	Total → MPA	4.98	30.20	20.78
	B→ TA	8.27	3.40	5.29
	SB→ TA	1.89	4.53	1.51
	Total → TA	10.16	7.93	6.80
Microscopic fungi [10^3 CFU.ml^{-1}]	MF→Cz-DA	154.88	79.31	105.71
	MF→MA	177.54	56.65	120.89
	Total	332.42	135.65	226.60

CFU - colony forming unit; B - forms of bacteria utilising organic nitrogen on the MPA and anorganic nitrogen on the TA; SB - spores of bacteria; MF -microscopic fungi on the Cz-DA or MA; C - control; MPA - meat-peptone agar, TA - Thorton agar; Cz-DA - Czapek-Dox agar; MA -malt extract agar; → S,B,MF on the definite agar

In the soil, increase of the total number of aerobic bacteria as well as their spores was determined which utilise organic nitrogen (meat-peptone agar) as a result of optimal moisture and temperature conditions during the incubation period (92 days). After polyethylene treatment, the increase of aerobic bacteria population discussed above was lower by one third, which lead to decreased CO_2 production. In both treatments, the prevalence especially those active forms of bacteria and their spore forms were observed that were able to utilise organic nitrogen (meat-peptone agar). This phenomenon was found to be in all soil treatments. The number of aerobically active bacteria (in Thorton´s agar) which use inorganic nitrogen, decreased at the end of experimental period. In the soil, increase of their spores was found, while in polyethylene amended soil, their number slightly decreased. The population density of microscopic fungi also decreased in both agar media at the end of the experimental period.

When compared with the row soil, addition of polyethylene caused reduction of bacteria as well as spore of bacteria number utilising the organic nitrogen after a 3-month lasting incubation period. There was determined also the increase of the inorganic nitrogen utilising active bacterial forms and the microscopic fungi, too.

The statistical evaluation of the obtained results of microbiological analysis of the soil amended with polyethylene showed non-parametrical distribution of the file (Table 1). For this reason, Kruskal - Wallis´s test was used once again. In accordance with this test (Table 2), the significant differences were found between the individual physiological microbial groups included bacteria and fungi. None significant differences were observed between the treatments and replicates (Table 2). High significant differences were determined between the bacteria on meat-peptone agar and microscopic fungi on malt extract agar. Based on this results, there was not proved significant effect of polyethylene on the soil microflora activity. Determined changes in CO_2 production as well as population densities of individual physiological microbial groups were caused by changing the soil properties during incubation period. The main reason seems to be reduction of O_2 capacity in the soil as a consequence of the granular polyethylene application into soil.

CONCLUSIONS

Among all tested samples, except of that including PE, increased soil respiratory activity was determined, which lead to increased mineralization of the organic substances. Both the hydrolysed

collagen containing polyvinylalcohol and the pure polyvinylalcohol are biodegradable by the soil microflora. In order to increase their mineralization, it seems as more effective to produce them with a higher content of additives. No effect was observed when the granular polyethylene was added into the soil as a recalcitrant compound, although surface contact with the soil was increased. In this case, the changes of physical soil properties as well as reduction of number of aerobic bacterial forms was found. During the three month lasting incubation test, no significant effect was determined on the reduction of the numerous representation of the microscopic fungi in the soil sample as well as in the sample amended with polyethylene.

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REFERENCES

[1] Albertsson A-CH. (1978): Biodegradation of synthetic polymers.II. A limited microbial conversion of ^{14}C in polyethylene to $^{14}CO_2$ by some soil fungi. Journal of Applied Polymer Science, *22*: 3419 – 3433

[2] Albertsson A-CH., Karlsson S. (1988): The three stages in degradation of polymers-polyethylene as a model substance. Journal of Applied Polymer Science, *35*: 1289 – 1302

[3] Albertsson A-CH., Ranby B. (1979): Biodegradation of syntetic polymers. IV.The $^{14}CO_2$ method applied to linear polyethylene containing a biodegradable additive. Journal of Applied Polymer Science, *35*: 423-430

[4] Alef K., Nannipieri P. (1995): Methods in applied soil microbiology and biochemistry. London, Academic press limited: 464-465

[5] Alexander M. (1994): Biodegradation and bioremediation. London, Academic press: 272-280

[6] Bejuki W.M. (1985): Microbiological degradation. Biosciences information service of biological abstracts, *2*: 220-241

[7] Bielek P., Matúšková L. (1998): Biologické vlastnosti znečistených a neznečistených pôd. Vedecké práce, *21*: 7-12

[8] Krupp L.R., Jewel W.J. (1992): Biodegradability of modified plastic films in controlled biological environments. Environ.Sci.Technol., *26*: 193-198

[9] Palmisano A.C., Pettigrew Ch.A.(1992): Biodegradability of plastics. BioScience, *42*: 680-686

[10] Paul E.A., Clark F.E. (1989): Soil microbiology and biochemistry. London, Academic press limited: 138-139

[11] Šantrůčková H. (1993): Respirace půdy jako ukazovatel její biologické aktivity. Rostlinná výroba, *39*: 769-778

[12] Standard method ASTM 5209 (1992): Standard test method for determining the aerobic biodegradation of plastic materials in the presence of municipal sewage sludge.

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