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Degradation of atrazine in soil TYPES with different characteristics

Ph.D. Thesis

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1. INTRODUCTION

Soil as a renewable natural resource has exceptional qualities: in addition to providing a place for the growth of natural and artificial crops it also integrates and transforms the effects of other natural resources. It is also capable of reducing the possible harmful effects of human activities. Soil may also be interpreted as a vast natural filter system, having the capacity to protect sub-surface levels and water resources from pollutants on the surface (Várallyay, 1992).

The quality of soil - meaning not only fertility, but also the ability to function in the manner mentioned above - can be remarkably varied. According to Stefanovits et al. (1999) there are 38 types of soil in Hungary, with additional 87 subtypes, each with different physical, chemical and microbiological characteristics.

Among the stress factors effecting soil, pollution related to agricultural activities is of increasing importance. This includes the use of fertilisers, soil improvement agents, and pesticides. Reasonable use without overdosing usually does not cause pollution, but unprofessional overuse may. These substances, besides being potentially toxic to plants and micro-organisms in the soil, may also enter the food chain, or, by filtrating into sub-surface levels, may also pollute surface and groundwater resources. Thus, it is extraordinarily important to study the processes these chemicals undergo, and their behaviour in different environments and in the case of different soil types (MANNINGER, 1967; KECSKÉS, 1985; THYLL, 1996).

Among pollutants containing organic chemicals used in relation with agricultural activities, soil disinfectants and herbicides are a primary issue, because these materials are delivered into the soil or onto the surface directly (HELMECZI et al., 1988). The popularity of atrazine containing herbicides in the use of corn production derives from their wide spectrum of activity, long-term effect, and excellent selectivity. Together with their low price, these characteristics have kept them on the market for almost 50 years (Helmeczi, 1983; KÁTAI and Helmeczi, 1991).

The use of atrazine as an active ingredient, and the maximum amount applicable at a time has been regulated by law in Hungary since as early as 1972, when it was fixed at a maximum amount of 3 kg/ha/2 years dosage. In 1995, the use of herbicides containing atrazine was re-licensed and the dosage was limited to 0.7-1.4 kg/ha of active ingredient.

According to a study performed in 1993, the most common herbicide in the upper layers of soil was atrazine (Aktinit PK) (KÁROLY et al., 1999). Due to the decline of herbicidal use, according to 1998 data, in nearly 68% of sub-surface waters, atrazine could not be detected, and only in 1% was it more than the 2 m g/l limit set by WHO (RAKICS, 2000, KÁRPÁTI et al., 1998). According to EU directives, the maximum allowance of atrazine concentration is 0,1 m g/l as for most pesticides (EC, 1998).

Although most of the atrazine delivered remains in the upper layers of the soil where it produces its effect (KÁROLY et al., 1991), it was also found in rainwater (RICHARDS et al.,1987; NATIONS and HALLBERG, 1992), and also in deposits produced by wind erosion (GLOTFELTY et al.,1989). In groundwater atrazine is the most often detected herbicide (RITTER 1990.).

The transport of atrazine to groundwater depends on a number of conditions including physical and chemical properties of the compound, the physical and chemical properties of the soil, and the amount and composition of organisms and their activities in the soil, together with other environmental factors.

The objectives of our study were:

- Adaptation of routinely applicable extraction and measurement methods to determine atrazine concentration in soil,
- verification of the method in a ring test,
- determination of soil properties in the profiles in three experimental fields,
- measurement of atrazine concentrations in the layers examined,
- setting up an incubation experiment to follow the degradation of atrazine,
- correlation analysis to study the relationship between the degree of atrazine degradation and soil properties.

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2. MATERIALS AND METHODS

2.1. Sampling sites

2.1.1. Experimental Farm in Pallag

The soil of the experimental field is **blown sand (sand mantle).** Horticultural crops have been grown in the area in previous years. Different dosages of fertilizers were applied with varied amounts of irrigation. No atrazine containing herbicides were used in the area.

2.1.2. Debrecen Experimental Farms of Regional Research Institute in Látókép

The soil type of the experimental ground is **calcareous chernozem**. In the last 15 years, corn has been grown on the sampling areas in continuous production. Between 1982 and 1988 atrazine containing herbicides were used at 2 kg/ha dosage. Due to the changes in the weed flora, they have applied combined materials since 1988, in which the ratio of atrazine containing materials was 20-30 %. As a result, the atrazine charge of the area has been significantly reduced in recent years. Different amounts of fertilizers and irrigation were applied in the area.

2.1.3. Experimental Farm in Hajdúböszörmény

The soil type of the area is **meadow soil**. Atrazine containing herbicides were only applied occasionaly in continuous corn production until 1992. Since 1992, atrazine has been used at 1.2 kg/ha/year dosage, combined with other herbicides.

2.2. Sampling and treatment of samples

Soil samples were taken in sterile conditions. In order to fully separate each soil layer, we used specially prepared steel cylinders, which were cleaned and sterilized in an autoclave before use.

At the sampling site a 2-meter deep hole was dug. The samples in the case of calcareous chernozem and meadow soil sample was taken every 20 cm, in the case of blown sand every 30 cm down to 190 cm, and below 190 cm every 30 cm with the sterile steel cylinders mentioned above. (In the case of blown sand, because of the morphological properties of certain layers, we did not manage to take the number of samples as planned. Only the centre, untouched part of the samples in the steel cylinders were used, which were placed into sterilized containers, and were stored in a refrigerator until use.

From 200-600 cm, samples were collected with an Eilkelkamp type drilling machine. In the case of calcareous chernozem and meadow soil, samples were taken from 200 to 300 cm by 25, from 300 to 600 cm by 50 cm. In the blown sand profile samples were collected from 190 to 240 cm by 25, from 240 to 600 cm by 50 cm. The planned sample amount was modified in some cases because of the morphological feature of the sampling site. To avoid contamination, the middle, untouched part of the soil cores were used for the experiments. In the meadow soil profile, groundwater was found at the depth of 5 meters.

2.3. Methods of analysis

Some physical, chemical characteristics of the soil samples were measured. The total number of bacteria was also determined.

Water content was measured by weight loss after drying at 105 °C and was expressed in the proportion of dry soil.

The pH value was determined both in distilled water and M KCl suspension.

Physical clay and silt, (particles less than 0.22 mm) were determined by elutriation.

Lime content was determined using the method from BALLENEGGER – di GLÉRIA (1962).

Organic carbon content was measured by SZÉKELY’s et al. (1960) method.

NO_3^- content of the layers was determined by Felföldy’s (1987) method.

Total number of bacteria was determined by plate dilution method on Tryptone soya agar medium.

Atrazine was measured with MERCK-HITACHI HPLC after extraction with a mixture of Acetonitrile : distilled water 9:1, using our own method.

The parameters of analysis:

HPLC system, D-7000 Chromatography Data Station Software, MERCK-HITACHI

Column: Lichrospher 100 RP-8 (5m m), 125 x 4 mm, MERCK

Eluent: Acetonitril: distilled water 70: 30.

Flow: 0,8 ml/min

Waveleght: 220 nm.

2.4.Degradation experiment

One series of the soil samples were autoclaved on three consecutive days (128° C, 2,5 bar, 60 min). The other series of the samples were used in the experiment in their original state.

One original and one autoclaved series belonged to all the three examined soil types.

To 6 x 50 g subsample from each layer 5-5 cm^3 of 200 mg/l atrazine was added. The thoroughly mixed samples were put into thermostat and the temperature was set to 28°C. During the incubation experiment, samples were taken after 28, 84 and 168 days in the case of calcareous chernozem, while after 28, 84 és 168 days in the blownsand and meadow soil. The amount of atrazine and the total number of bacteria were checked in the samples. After taking samples, the mass was recorded and at the beginning of the next sampling the lost water (sterile) was added.

As described previously, every result of atrazine content and total number of bacteria was calculated as an average value of six data.

Our methods were validated in a ring test. Four participant teams sent one surface and one sub-surface sample to the co-ordinator. After distributing samples, all of the participant laboratories performed incubation experiments as described above, with four sampling and analysis.

The original aim was to prepare sterile soil samples. The control of the samples proved that viable micro-organisms were found even after three consecutive autoclaving processes. Therefore, in our degradation experiments we used soil samples with reduced number of bacteria and in the thesis we mention “original” and “autoclaved” but not “sterile” soil samples.

For processing the data of the experiments an IBM compatible computer was used with Microsoft Windows 98 operation system. Correlation between different data was calculated by regression analysis.

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3. RESULTS

3.1. Horticultural Experimental Farm, Debrecen-Pallag

Horticultural crops have been grown in the area in previous years. Different dosages of fertilizers were applied with varied amounts of irrigation. No atrazine containing herbicides were used in the area.

3.1.1. Physical, chemical and microbiological characteristics of the soil

The soil type of the experimental farm blown sand, the subtype is sand mantle. Some physical, chemical characteristics and the total number of viable bacteria are shown in Table 1.

The water content of the layers at the sampling time was relatively low. The smallest values were measured in the layers 160-290.

Clay and silt content slightly increased to 160 cm, between 160-290 cm the values were lower, and there was a considerable increase in the deepest layers.

pH values both in distilled water and M KCl suspension increased down to 160 cm, but there was no considerable change from there down to the bottom.

$CaCO_3$ content appeared at 100 cm and varied between 2,5 and 9,7 %.

Organic matter content decreased gradually from the top to 130 cm, and its amount was very small in the deeper layers.

The nitrate content of the samples indicated accumulation in the layers between 50-130 cm.

The total number of bacteria proved to be relatively high not only in the surface layers, but also between 400 and 600 cm. We could detect an especially high value in layer 400-450 cm.

There was no detectable amount of atrazine in the layers of the profile.

Table 1. Characterization of soil samples
blownsand, Debrecen-Pallag, 1996. 04.29.)

Layer (cm)	Water content	Clay and silt	$pH(H_2O)$	pH(M KCl)	CaCO3	Organic carbon	NO_3^-	Total nr. of

	(%)	(%)			(%)	%	(mg/kg)	bact. (mill/g soil)
0-30	10.99	12.60	6.82	6.10	0	0.48	13,2	8,39
30-50	11.86	12.92	6.40	5.40	0	0.63	8,9	2,87
50-70	11.76	13.24	6.70	5.80	0	0.36	21,4	2,93
70-100	9.95	14.04	7.10	6.40	0	0.20	11,5	1,67
100-130	9.31	15.48	8.20	7.85	4.2	0.16	6,0	2,27
130-160	9.12	15.64	8.20	8.01	9.7	0.06	4,5	3,52
160-190	7.23	10.36	8.28	8.02	5.9	0.04	5,7	0,88
190-215	7.74	11.20	8.16	7.98	6.5	0.05	4,7	1,50
215-240	7.78	8.04	8.22	7.92	6.9	0.03	2,5	1,71
240-290	6.72	6.96	8.32	7.92	2.5	0.04	2,9	1,59
290-350	10.89	17.04	8.30	7.78	6.8	0.04	6,2	1,47
350-400	17.01	28.12	8.30	7.78	8.4	0.09	1,6	2,48
400-450	13.13	34.00	8.20	7.68	5.7	0.09	1,1	10,1
450-500	21.60	24.28	8.25	7.65	5.7	0.09	1,8	7,0
500-550	17.65	29.35	8.18	7.65	5.1	0.11	1,0	5,1
550-600	24.27	34.36	8.30	7.68	5.1	0.21	13,2	5,2

Figure 1

3.1.2. The results of the degradation experiment with blown sand

The results of the incubation experiment (changing of atrazine concentration in the layers during incubation, considering the atrazine concentration at start as 100 %.) are shown in Figure 1.

As it can be seen in figure, except the upper layers, there is very little difference in the concentration of the detectable atrazine after 28 and 56 days of incubation between the original and autoclaved samples. Essential difference can be seen only after 112 days, mainly in the layers 0-130 and 450–600 cm. It is remarkable, that much more atrazine remained in the samples of autoclaved series than in the original ones.

The biggest atrazine dissipation was found in those layers, where the highest clay and silt content and highest number of bacteria was detected.

3.2. Experimental Farm of Regional Research Institute Debrecen-Látókép

In the last 15 years, corn has been grown on the sampling site in continuous production. Between 1982 and 1988, atrazine containing herbicides were used at 2 kg/ha dosage. Due to the changes in the weed flora, they have applied combined materials since 1988, in which the ratio of atrazine containing materials was 20-30 %. As a result, the atrazine charge of the area has been significantly reduced in recent years. Different amounts of fertilizers and irrigation were applied in the area.

3.2.1. Physical, chemical and microbiological characteristics of the soil

The soil type of the experimental ground is calcareous chernozem. Some physical, chemical characteristics and the total number of viable bacteria are shown in Table 2.

The water content of the layers at the sampling time varied between 19,9 and 24,4%.

Clay and silt content altered between 40,03 and 43,04 in the upper 120 cm layers, gradually decreased to 225 cm, in the deeper layers varied from 37,2 to 39,35 %.

PH values both in distilled water and M KCl suspension increased down to 140 cm, but there was no considerable change from there down to the bottom.

$CaCO_3$ content appeared at 120 cm and varied between 2 and 6 %.

Organic matter content decreased gradually from the top to 160 cm, and hardly changed in the deeper layers.

The nitrate content of the samples indicated accumulation in the layers between 180-350 cm.

The total number of bacteria gradually decreased to 140 cm but there were very small changes in the deeper layers.

3.2.2. Extractable atrazine content of the soil.

The extractable atrazine content in the layers of the profile was very low, and gradually decreased down to 160 cm and hardly changed to the bottom of the profile. There was no accumulation detected in the profile.

Table 2. Characterization of soil samples
(calcareous chernozem, Debrecen-Látókép, 03. 26.1996.)

Layer (cm)	Water content (%)	Clay and silt (%)	$pH(H_2O)$	pH(M KCl)	CaCO3 (%)	Organic carbon %	NO_3^- (mg/kg)	Total nr. of bact (mill/g soil)
0-20	23.30	43.04	6.30	5.90	0	1.27	9,8	8,95
20-40	23.32	42.80	6.75	6.30	0	1.13	9,6	6,33
40-60	22.62	42.32	6.85	6.25	0	1.08	7,0	1,85
60-80	21.58	41.40	7.15	6.50	0	0.73	5,0	1,73
80-100	21.07	40.03	7.20	6.60	0	0.53	2,2	1,13 130,85
100-120	19.90	40.88	7.30	6.75	0.01	0.38	2,4	0,85
120-140	21.43	38.00	7.60	7.60	5.73	0.26	9,9	0,47
140-160	20.26	34.88	7.65	7.75	5.16	0.19	4,3	0,23
160-180	20.26	32.68	7.55	7.80	5.44	0.19	7,0	0,20
180-200	21.14	31.28	7.55	7.85	6.01	0.13	13,1	0,31
200-225	20.48	29.68	7.80	7.80	5.30	0.13	17,6	0,20
225-250	20.70	32.32	7.85	7.85	4.01	0.13	17,3	0,21
250-275	22.10	34.10	7.70	7.80	3.58	0.19	20,0	0,22
275-300	22.93	39.35	7.60	7.70	2.72	0.19	22,9	0,118
300-350	23.76	38.90	7.55	7.70	2.00	0.26	12,2	0,296
350-400	21.21	39.24	7.70	7.70	4.30	0.21	1,9	0,033
400-450	21.35	37.52	7.80	7.80	4.15	0.13	0,6	0,155
450-500	23.15	38.04	7.80	7.90	4.30	0.06	4,1	0,096
500-550	24.22	39.60	7.75	7.80	5.16	0.14	5,0	0,265
550-600	21.51	37.52	7.75	7.80	4.30	0.13	5,9	0,178

Figure 2

3.3. Experimental Farm, Hajdúböszörmény

The soil type of the area is meadow soil. Atrazine containing herbicides were only applied occasionaly in the continuous corn production until 1992. Since 1992 atrazine has been used at 1.2 kg/ha/year dosage combined with other herbicides.

3.3.1. Physical, chemical and microbiological characteristics of the soil

Some physical, chemical characteristics and the total number of bacteria are shown in Table 3.

The water content of the layers varied between 15.66-27.24%. The smallest value was measured at 140-160 cm.

Clay and silt content was very high between 0-40 and 160-250 cm, but decreased considerably from 350 cm.

pH value measured in distilled water an M KCl suspension slightly increased from 0 to 40 cm, while due to the appearing $CaCO_3$ there was a considerable increase from 40-60 cm.

Organic matter content gradually decreased down to 60 cm and there was no change from there to the bottom of the profile.

In the layers 60-140 cm nitrate accumulation was detected.

The total number of bacteria gradually decreased in layers down to 140 cm and there was no considerable change in deeper layers.

3.3.2. Extractable atrazine content of the soil

The extractable atrazine content of the samples was determined (Figure 3.). After relatively low levels in the upper 140 cm layers remarkable accumulation was found between 250 and 500 cm. The maximum value (0.53 mg/kg) was found at 225-250.

3.3.3. The results of the degradation experiment with meadow soil

The results of the incubation experiment (changing of atrazine concentration in the layers during incubation, considering the atrazin concentration at start as 100 %.) are shown in Figure 4.

The atrazine dissipation during the first 28-day incubation proved to be very similar in the 0-20 cm layer both in the original and the autoclaved samples. Between 20 and 60 cm more atrazine remained in the original samples. The atrazine content of the original samples below 60 cm slightly decreased compared to the start. Higher rate of dissipation was detected after 56-day incubation in the upper 0-40 cm and in the bottom 400-500 cm layers. The atrazine content in the autoclaved samples from 40 to 500 cm practically have not changed. Atrazine content decreased in the original samples bigger rate of dissipation was found in layers 0-20 cm and 400-500 cm, compared to the previous sampling time. In the original samples between 400 and 500 cm 13.8 and 13.5 %, while in the autoclaved samples 69.8 and 80.5 % of the start value was found after 112 days of incubation.

Table 3. Characterization of soil samples
(meadow soil, Hajdúböszörmény, 04. 24. 1996.)

Layer (cm)	Water content (%)	Clay and silt (%)	$pH(H_2O)$	pH(M KCl)	CaCO3 (%)	Organic carbon (%)	NO_3^- (mg/kg)	Total nr. of bact. (mill/ g soil)
0-20	25.36	65.76	6.60	5.78	0	2.44	10,7	15,24
20-40	26.15	62.76	6.85	6.12	0	1.69	11,7	7,42
40-60	21.54	45.80	8.02	7.48	12.7	0.46	21,6	4,91
60-80	21.71	43.36	8.13	7.58	17.7	0.20	19,8	2,94
80-100	21.86	51.52	8.08	7.62	24.9	0.17	71,9	1,12
100-120	18.23	47.04	8.05	7.60	12.7	0.15	90,7	0,548
120-140	15.86	48.20	8.12	7.63	11.8	0.16	53,9	0,364
140-160	15.66	57.96	8.25	7.60	9.3	0.19	27,7	0,185
160-180	17.76	71.48	8.27	7.42	2.5	0.20	15,7	0,124
180-200	21.98	66.48	8.15	7.15	0.0	0.19	11,2	0,829
200-225	23.76	70.04	8.30	7.55	5.9	0.36	4,4	0,318
225-250	27.16	62.92	8.42	7.55	10.1	0.31	2,7	0,159
250-275	27.24	53.68	8.40	7.52	3.8	0.17	0,2	0,477
275-300	23.05	46.08	8.32	7.45	0.8	0.09	1,3	0,105
300-350	22.04	54.64	8.35	7.60	5.1	0.19	2,6	0,155
350-400	20.38	21.56	8.32	7.62	0.4	0.07	1,3	0,139
400-450	21.89	22.28	8.32	7.78	0.0	0.07	0,2	0,481
450-500	18.53	8.52	8.30	7.62	0.0	0.02	0,2	0,574

Figure 3.

Figure 4.

3.4. Correlation analyses

Regression analysis between soil characteristics of the blown sand in Debrecen-Pallag and the quantity of dissipated atrazine in original samples show strong correlation between dissipated atrazine and clay and silt content (r= 0.76***) and the total number of bacteria (0.79***)

In the case of the autoclaved series of samples there was also a relationship concerning the clay and silt content. The value of correlation coefficient in the equation used to describe the relationship is lower in this case (**r= 0.61****).

There was no correlation in the case of the original and autoclaved samples of **calcareous chernozem profile** layers. There is no remarkable difference between the levels of degradation in the original and autoclaved samples. From the correlation analysis we can conclude that atrazine degradation in the case of this soil type is not dominated by biological processes. (The relationship between the number of bacteria and the percentage of atrazine degraded is **r = 0.29**).

Based on the correlation analyses between the soil characteristics and atrazine degradation we may conclude that in the case of the original samples only clay and silt content show close correlation with the amount of degraded atrazine (**r=0.77*****).

Correlation analyses between the soil characteristics of meadow soil and atrazine degradation show that there was strong correlation between the number of bacteria and the amount of dissipated atrazine in the case of the original sample series (**r=0.82***). There was** no significant correlation in the case of clay and silt content (**r=0.59****). In the autoclaved series strong correlation was found between the amount of dissipated atrazine and organic carbon (**r=0.93*****) or the pH values (**r=0.95*****).

The results of the correlation analyses are shown in Table 4.

Table 4. Correlation between the amount of dissipated atrezine and the soil characteristics

Soil characteristics	Dissipated atrazine %					
	Original soil			*Autoclaved soil*		
	Blown sand	Calcareous chernozem	Meadow soil	***Blown sand***	*Calcareous chernozem*	*Meadow soil*
Organic Carbon %	0,04	0,47	0,35	*0,01*	*0,49**	***0,93****
Clay and silt %	**0,76*****	**0,77*****	**0,59****	***0,61****	*0,31*	*0,21*
pH (H_2O)	-	-	-	*-*	*-*	***0,95*****
$CaCO_3$ %	0,26	0,46*	0,48*	*0,31*	*0,36*	*0,23*
NO_3^- mg/kg	0,36	0,3	0,23	*0,34*	*0,19*	*0,33*
Total number.of bact.	**0,79*****	0,29	**0,82*****	***0,67****	*0,48**	***0,78*****

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4. New and novel scientific results

1. The occurrence and accumulation of atrazine and its metabolites indicate a serious ecological and environmental threat. In spite of the reduced use, the transformation of atrazine should be followed by regular analyses.
2. The physical and chemical characteristics of soils fundamentally influence the transformation, transport, sorption and degradation of atrazine introduced to the soil. Texture and water content among physical, pH and organic compound content among chemical characteristics should be highlighted as most significant.
3. The accumulation of atrazine in a certain soil type depends on several factors. On the basis of our results we can state that atrazine accumulation formed in those soil layers where the conditions were not favourable for the occurrence and multiplication of bacteria.
4. In our experiment we could prove that in the case of blown sand, calcareous chernozem and meadow soil microorganisms played a significant role in atrazine degradation even in sub-surface layers, where there was not supposed to find microbiological activity earlier.
5. In the case of two soil types - blown sand (sand mantle) and meadow soil – a mathematically verified correlation was found between the ratio of degraded atrazine and the total number of bacteria in the soil in the incubation experiment
6. Faster atrazine degradation was determined in the surface compared to the sub-surface layers. In our experiment it was verified that the half-life of atrazine was shorter in the surface than in the sub-surface layers of soil.

Publications related to the subject of dissertation

1. REVIEWED PUBLICATIONS:

Issa, S. - Wood, M - Pussemier, L - Vanderheyden, V. - Douka, C. - Vizantinopoulos, S. - Győri, Z. - **Borbély, M.** - Kátai, J. (1997): Potential dissipation of atrazine in the soil unsaturated zone: a comparative study in four countries. Pesticide Science, 50., 99-103. p.(0,8)

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Borbély, M. - Győri, Z. - Kátai, J.(1997): Az atrazin lebomlása egy réti talaj profiljában. Tiszántúli Mezőgazdasági Tudományos Napok, Karcag 1997. Június12-13. (poszter) 268-269. p.

KÁTAI, J.- **BORBÉLY, M**.- GYŐRI, Z. (1999a). Atrazinbontás tanulmányozása egy nemzetközi projekt körtesztjének keretében. DATE Tudományos Közleményei Tom. XXXIV. Debrecen. (0,1)

KÁTAI J.- **BORBÉLY M**.- GYŐRI Z. (1999b): Összefüggések az atrazin-degradáció és a talaj fontosabb tulajdonságai között. Debrecen, Tiszántúli Mezőgazdasági Tudományos Napok. Talajtan és Agrokémia sz. p. 93- 102. (0,1)

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2. POSTERS PRESENTED IN INTERNATIONAL CONFERENCES:

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