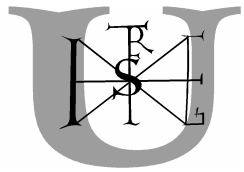

SZENT ISTVÁN UNIVERSITY

HERBICIDE-SENSITIVITY OF SOME MICROORGANISMS ON THE BASIS OF THE CHLORSULFURON

Ph.D thesis

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INTRODUCTION

Intensification of crop production practices has enhanced the herbicide use in the agriculture. Apart from the desired toxic effects of some herbicides on weeds, however, the treatments may have a number of possible unfavourable „side effects" on organisms and may cause soil pollution with toxic materials. An outstanding tendency nowadays is the development of a new herbicide family with less harmful effects in connection with a reduced applied concentration. So it is the current task nowadays in the agricultural chemistry to apply chemicals, which are less toxic on the soil microorganisms, the ecosystems, the human health and for the animals. The main requirements for the herbicides are the long-lasting herbicide effects, and also the ability of degradation, the non toxic degradation products and small application doses.

World Congress of UNO Environmental Protection nominated the pesticide compounds as the ten most dangerous pollutants, due to the fact that they are polluting not only the agricultural soils but also the air, the surface and the underground waters, as well. So the pesticides might threaten the environmental balance and stability of the World. The over expressed chemisation of the agriculture might alter the natural components of the soils and their functioning (Stefanovits 1977).

Several environmental factors might affect the growth of microorganisms in the soils. Among the agricultural chemicals the herbicides are known as the most frequently used. Chlorsulfuron as a member of the “new generation” herbicides (with a sulfonylurea content) is generally efficient in the weed control of grain crops, linum and soybeans. Although a 2-fold lower rate is recommended in the agriculture in comparison with conventional herbicides, the chlorsulfuron can be still 100 times more efficient against the weeds, but can be harmful for the soil microbes.

The sensitivity of the microorganisms might be various in the soils. The structure or the function of microbial communities might be influenced by the herbicide applications especially on the basis of the long-term periods. According to the sustainable agriculture and the environmental protection way of thinking it is necessary to study the herbicide-sensitivity of different microorganisms, their tolerance limits, adaptation ability to the agricultural chemicals and the relationship between their functioning. It seems to be also a key-issue to find appropriate methods for detecting the microbiological effects of the herbicides.

The „new-generation” herbicide chlorsulfuron and the “old-style” thiocarbamate-herbicides were examined on the growth of some soil microorganisms, genus, strains and groups (important as key-microbes in soil-functioning) among the laboratory conditions *in vitro* and also in soil-incubation model experiments *in vivo*.

Objectives

Study on the microbial sensitivity to the various doses of some herbicides *in vitro* and *in vivo* soil-incubation model-experiments, so as to analyse the growth sensitivity of several typical and characteristic microorganism groups, genus and strains isolated from soils and/or soil-plant systems. To examine

- the sensitivity patterns of several microorganism genus and strains, through the differences in their growth,
- the sensitivity differences of the microbial growth regarding a single microbial genus,
- the dose- and time-dependent effects of herbicides for the abundance of microorganisms, by using "practical" and over-estimated concentrations for the microbial growth,
- the methodological opportunities in assessing the microbial sensitivities,
- the abundance of specific microorganisms at short affecting periods of herbicide doses and after the longer (i.e. the vegetation) period,
- the effects of the chlorsulfuron in comparison with other thiocarbamate-type herbicides,
- the interaction of herbicides and the industrial waste water, as one of the environmental factors influencing the herbicide application,

that from all these results we can make potentially useful conclusions for the practical applications.

MATERIALS AND METHODS

I. *In vitro* methods to study the herbicide-sensitivity of microorganisms

The following microorganisms and microbial-groups were examined among the laboratory conditions:

1) N_2-fixing bacteria: ***Azotobacter*** spp., ***Rhizobium*** *leguminosarum* bv. *viciae* Bük-75/4, *R. leguminosarum* bv. *trifolii* Ló-73/3, ***Bradyrhizobium*** *(Lupinus)* sp. Csf-75/1, ***Sinorhizobium*** *meliloti* Lu-K;

2) Spore-forming bacteria: ***Bacillus*** *cereus* var. *mycoides* and *B. subtilis;*

3) *Streptomycetes*: ***Streptomyces*** *griseus, S. griseolus*;

4) *Pseudomonads*: ***Pseudomonas*** *aeruginosa, P. alcaligenes, P. fluorescens;*

5) Potential pathogens: ***Agrobacterium*** *tumefaciens,* ***Erwinia*** *carotovora,* ***Escherichia*** *coli,* ***Micrococcus*** *luteus* and ***Xanthomonas*** *campestris*.

The sensitivity of microbial growth to the different rates of sulfonylurea herbicide chlorsulfuron (**Figure-1**) was examined by some *in vitro* methods.

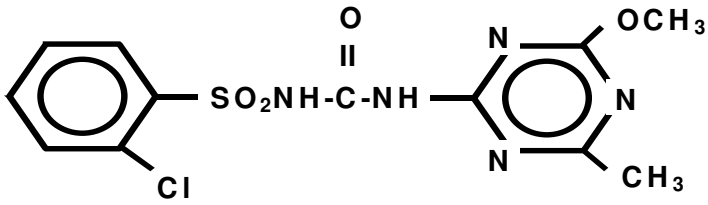
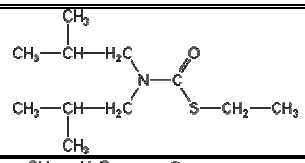
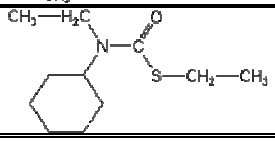
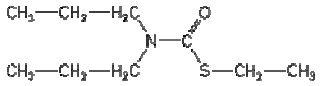
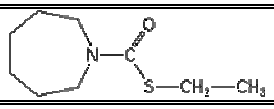
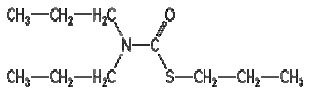


Figure -1: Chemical structure of the chlorsulfuron (2-chlor-/N/4-methoxy-6-methyl-1,3,5-triazine-2-il/-aminocarbonyl/-benzosulfonamide)

To understand more accurately of the herbicide-sensitivity of the microorganisms an *in vitro* experiment were done with herbicides of older-generation but previously widely applied thiocarbamates (butylate, EPTC, cycloate, molinate, vernolate) by using increasing concentrations (Table 1).

Table 1: Some characteristics of used thiocarbamate herbicides

Thiocarbamate herbicides	Trade name	Chemical name	Structure
Butylate	Anelda 72EC	S-ethyl-N,N-diizobuthyl-thiocarbamate	
Cycloate	Sabet 72EC	S-ethyl-N-ethyl-N-cyclohexyl-thiocarbamate	
EPTC	Witox 72EC	N,N-di-n-propyl-S-ethyl-thiocarbamate	
Molinate	Molinate	S-ethyl-N,N-hexamethylene-thiocarbamate	
Vernolate	Vernolate	S-n-propyl-N,N-di-n-propyl-thiocarbamate	

The *in vitro* sensitivity of various soil microbes to the chlorsulfuron and to some thiocarbamate herbicides was examined in a rotary shaker, where different rates of chlorsulfuron (0.001-, 0.01-, 0.1-, 1-, 10 mgL^{-1}) and thiocarbamates (50-, 100-, 250-, 500-, 1000 mgL^{-1}) were mixed into liquid selective substrates /Nutrient (N), Yeast-Extract. Mannite (YEM) and an Arginine-Glycerine Broth

(AGB). Most of the microorganisms are growing on the Nutrient substrate, except of the N_2-fixers and the *Actinomyces,* where the YEM and the AGB were used, respectively. After a 24-hours of incubation period (or 72-hours for the Actinomycetes), the optical density (OD) (for AG-530-, for YEM-560- and for Nutrient-640 nm) of the suspensions was examined and growth was compared as a % of the unamended controls. The dry matter content of *Streptomyces griseus* also was examined with and without herbicides. The counting plate method was used as a control. The results were evaluated by analysis of variance and the significant differences were shown beside the mean values.

II. Soil-incubation microcosm experiments *in vivo*

A soil incubation model experiment was set up to study the effect of recommended rates (10-20 gha^{-1}) one of a new generation of herbicides chlorsulfuron, on microbial communities in a pseudomyceliar chernozem soil [$pH_{(H2O)}$–7,7, $pH_{(KCl)}$–7,0; organic matter–3,6 %, $CaCO_3$–3,27 %; ingredients ($mgkg^{-1}$): N–1692, P–1690, K–1190, Ca–5958, NH_4N–3,6; NO_3N–32,0 (Bremner), Al-P_2O_5–123, Mg–140, Al-K_2O–194, Cu–1,87, Zn–0,73, Mn–71,3, Fe–1993 (EDTA)]. Apart from the recommended rate (equivalent to 0.001 $mgkg^{-1}$ soil), 10-, 1000-times this rate (0.01, 1 and 10 $mgkg^{-1}$) were also tested. Incubation periods of 3 weeks and 3 months at 28 °C and 60 % field moisture capacity were tested in three replicates. Combination of these herbicides was developed by the permanent watering of the pots with an industrial waste water (pH–7,5; KOI–3690-; phenol–546,7 mgL^{-1}; PAH–10 μgL^{-1}, BTEX: n.d., rodanide–346,6-; free cyanide–3,4-; total cyanide–9,5-; Kjeldahl N–350-; organic N–200-; nitrate-N–400-; ammonia–480-; total N–1000-; H_2S–115,6- mgL^{-1}), up till the 60 % water-capacity level. Free-living nitrogen fixers, *Actinomycetes*, spore-forming *Bacillus cereus* var. *mycoides* and heterotrophs were counted on arginine-glycerine, Congo-red Ashby and nutrient agar plates, respectively (Szegi 1979, Vincent 1970). Abundance of soil microbes was determined from a soil dilution series using a modified standard technique and number of colony-forming units was converted to 1 g of dry soil. Number of *B. cereus* var. *mycoides* was controlled by microscope. The degradation rate of chlorsulfuron was calculated by Thirunarayanan et al. (1985).

Simplified method of plate counting: dilution series was prepared from 1 g of soil by a parallel measure of the water content. Droplets of 20 µl suspensions were put onto the agar surface in three replicates, allowing the study of four different dilution steps in one Petri dish (Angerer et al. 1998, 2006, 2007, Biró and Angerer 1997). Selective media, as the Ashby- or Martin agar were used to count the colony-forming units (CFU g^{-1} dry soil) of the microbial groups of hetero-/oligotrophs, spore-formers, Actinomycetes, free-living N_2-fixers etc.). After the appropriate incubation the microbial counts as $\log_{10}$ values, were converted to 1 g. of dry soil and $LSD_{5\%}$ were also calculated by a variance analysis, indicating the least significant differences (at the 5 % level) (Reichart 2005).

RESULTS AND DISCUSSION

I. Herbicide sensitivity (growth) of microorganisms *in vitro*

Microorganisms tested *in vitro* showed a diverse sensitivity pattern to the examined rates of „new-generation" chlorsulfuron herbicide and also to the older type of thiocarbamates. Those differences in microbial growth based on the chemical structure of the herbicides, and the individual sensitivity of the microorganism groups/genus/strains. Analysing the dose-effect of herbicides generally the rates, which are applied in the agricultural practice (0.001 mgL^{-1}) and it tenfold amounts (0.01 mgL^{-1}) stimulated the bacterial growth. Significant microbial growth-inhibition could be observed as an average, at the 0.1- 1- and 10 mgL^{-1} chlorsulfuron doses.
Results of individual sensitivity of microorganisms are shown in the Table 2.

Table 2: Evaluation of chlorsulfuron sensitivity of microorganisms *in vitro*

Microorganisms	Chlorsulfuron doses				
	1	2	3	4	5
N_2-fixing bacteria					
Azotobacter spp.	+	+	0	0	-
Rhizobium *leguminosarum* bv. *viciae* Bük-75/4	+	+	-	-	-
R. *leguminosarum* bv. *trifolii* Ló-73/3	-	-	-	-	-
Sinorhizobium *meliloti* Lu-K	-	-	-	-	-
Bradyrhizobium *(Lupinus)* sp. Csf-75/1	-	-	-	-	-
Spore-forming bacteria					
Bacillus *cereus* var. *mycoides*	+	+	0	+	+
B. *subtilis*	-	-	-	-	-
Streptomyces					
Streptomyces *griseolus*	+	+	+	+	+
S. *griseus*	+	+	-	-	ND
Pseudomonas					
Pseudomonas. *alcaligenes*	+	+	+	+	+
P. *fluorescens*	+	+	+	+	+
P. *aeruginosa* *	+	-	-	-	-
Potential pathogens					
Agrobacterium *tumefaciens*	+	-	-	-	-
Erwinia *carotovora*	0	0	-	-	-
Escherichia *coli*	+	+	-	-	-
Xanthomonas *campestris*	-	-	-	-	-
Micrococcus *luteus*	+	+	-	-	-

Legend.: 0: no effect, +: significant stimulation, -: significant inhibition, -/0: no growth. ND: no data. Chlorsulfuron doses: 1) 0,001-; 2) 0,01-; 3) 0,1-; 4) 1,0-; 5) 10 mgL^{-1}.

Among the symbiotic nitrogen-fixing-microorganisms tested, the *Rhizobium leguminosarum* bv. *trifolii* strain Lo-73/3 and the *Sinorhizobium meliloti* strain Lu-K proved to be the most sensitive *in vitro* on chlorsulfuron, and growth was retarded in parallel with the increasing doses of this herbicide. The lowest rates of chlorsulfuron significantly stimulated the growth of *Streptomyces griseus*. The growth stimulation of *Streptomyces griseolus* and the *Pseudomonas alcaligenes* (135 %) and *P. fluorescens* (151 %) were found by the chlorsulfuron doses. According to literary data the *S. griseolus* has a chlorsulfuron decomposition ability. Certain rates of the herbicide also stimulated the multiplication of *B. cereus* var. *mycoides.* The *Xanthomonas campestris* on the other hand proved to be the most sensitive microorganism on every type of herbicides, resulting a 0-31 % of growth inhibition.

Thiocarbamates had more harmful effect on microbes than the chlorsulfuron. The *X. campestris, B. subtilis, B. mycoides, S. griseus, S. griseolus, M. luteus, S. meliloti* Lu-K, *E. coli, A. tumefaciens, E. carotovora, P. fluorescens* and *P. alcaligenes* were found to be very sensitive on thiocarbamate herbicides. The molinate was detected to be the most harmful among examined herbicides. The sensitivity of microorganisms has been strongly dose- and chemical-structure depended.

II. Chlorsulfuron sensitivity (abundance) of microorganisms in the soil-incubation model- experiment *in vivo*

There were a shorter- (3 weeks) and a longer- (3 months, similar to the vegetation) period, applied in the soil-incubation experiment. The growing rates of the various microbial groups to the increasing chlorsulfuron doses are shown in the Table 3.

Table 3: Abundance of some microbial groups *in vivo* at increasing doses of chlorsulfuron, after a shorter (3 weeks) and a longer (3 months) incubation periods

Microbial groups and affecting time	Chlorsulfuron doses			
	1	2	3	4
Free-living N_2-fixers				
3 weeks	**-**	**-**	**-**	**-**
3 months	**+**	**0**	**0**	**-**
Actinomycetes				
3 weeks	**0**	**0**	**0**	**-**
3 months	**+**	**+**	**+**	**-**
Spore-forming (Bacillus cereus var. mycoides)				
3 weeks	**0**	**0**	**0**	**0**
3 months	**0**	**-**	**-**	**-**
Heterotrophs				
3 weeks	**-**	**-**	**-**	**-**
3 months	**0**	**0**	**-**	**-**

Legend: 0: no effect, +: significant stimulation, -: significant inhibition. Chlorsulfuron doses:1) 0,001-; 2) 0,01-; 3) 1,0-; 4) 10 $mgkg^{-1}$

The soil incubation experiment has proved that chlorsulfuron concentrations higher than the practical rates has resulted significant reduction in the abundance of heterotroph microorganisms. Free-living nitrogen fixers were found to be the most sensitive microbes and *Actinomycetes* also had poor tolerance of some rates. Nevertheless, the field rate of 0.01 $mgkg^{-1}$ has a stimulating effect on several types of microbes. There were no significant differences between the control and the recommended rates in the colony-forming units after the 3 months of incubation, equivalent with the length of the vegetation period. It is assumed that the microbes are able to utilise the field rates of chlorsulfuron as carbon and nitrogen sources. Results of sensitivity of examined microbial groups to chlorsulfuron and wastewater combinations are shown in the Table 4.

Table 4: Abundance of some microbial groups after a shorter- (3 weeks) and longer (3 months) periods of chlorsulfuron and wastewater application groups *in vivo*

Microbial groups and affecting time	Chlorsulfuron doses with wastewater				
	1	2	3	4	5
Free-living N_2-fixers					
3 weeks	-	-	-	-	-
3 months	+	+	0	0	-
Actinomyces					
3 weeks	0	0	0	0	0
3 months	-	0	0	-	-
Spore-forming (Bacillus cereus var. mycoides)					
3 weeks	0	0	0	-	0
3 months	0	+	-	-/0	-/0
Heterotrophs					
3 weeks	+	0	+	0	0
3 months	+	+	0	-	-

Legend: 0: no effect, +: significant stimulation, -: significant inhibition, -/0: no growth, detected. Chlorsulfuron rates with waste-water: 1) 0, only wastewater; 2) 0,001-; 3) 0,01-; 4) 1,0-; 5) 10 $mgkg^{-1}$

There was a variable sensitivity found at the investigated countable microbial groups, as a function of the applied doses of the herbicide-wastewater combinations. Among the microbes the free-living nitrogen-fixers proved to be the most sensitive, which supports the earlier findings. In case of the herbicide-waste-water combined applications a modified effect could be realised over the single herbicide doses. Due to the cyanide-content of the used wastewater, there was a reduced microbial count realised, especially at the highest herbicide combinations. In case of the 0,01 $mgkg^{-1}$ chlorsulfuron doses, however the growth stimulation of the *Actinomycetes* and the *Bacillus cereus* var. *mycoides* could be also detected.

Due to the different sensitivities of the various microbial groups *in vitro* and *in vivo*, the microbial composition structure in the chlorsulfuron-amended soil may be shifted by the regular use in the agriculture. The consideration of such

microbial shifts, when designing the application of the different xenobiotics in the agriculture is being highlighted in this study.

CONCLUSION

The *in vitro* tests and the modified plate counting method were potentially applicable to study the microbial abundance *in vitro* and *in vivo*. According to those tests, applied the N_2-fixing bacteria was found to be the most sensitive microbes among the studied ones. Due to the different behaviour of the various microbial groups, investigated, the shift of the microbial composition structure can be concluded at the increasing doses of the chlorsulfuron herbicide application. Due to the changes of microbial abundance after a longer–incubation-period, the importance of a more frequent monitoring is recommended in the agro ecosystems. Using the simplified plate counting method a more frequent monitoring is possible on the main, beneficial soil microbes in connection with the effect of various environmental pollutants.

NEW SCIENTIFIC RESULTS

The herbicide sensitivity (i.e. the effect of herbicides) of the growth of different microorganisms of soil-plant systems was examined in laboratory conditions *in vitro* and in microcosm soil-incubation model experiment *in vivo.* Increasing rates of the „new-generation" herbicide chlorsulfuron and some „old-type" thiocarbamate herbicides were applied. In the soil-incubation experiment the chlorsulfuron and combination of industrial waste water with high organic matter- and toxic element contents were used. The short- and longer time-period effect also were examined.

A) Results of *in vitro* experiments:

1.) The practical dose of the „new-generation" chlorsulfuron herbicide (0,001 mgL^{-1}) and the 10-fold-, 100-fold- rates (0,01- 0,1 mgL^{-1}) stimulated the growth of microorganisms. The 10 mgL^{-1} herbicide-dose caused also significant growth stimulation of 2 *Pseudomonas*, 1 *Actinomyces* and 1 *Bacillus* strains. Such a growth stimulation is not characteristic for the „old-type" thiocarbamate herbicides, due to their higher rates of application and their variable chemical structures.

2.) The 17 strains representing 5 examined microbial groups showed individual reproduction sensitivity against the rates of chlorsulfuron and thiocarbamates. Among the microbes the nitrogen-fixers proved to be the most sensitive and according to the literary data, the known herbicide-degrading microbes, as the *Pseudomonas* and *Streptomyces* genus were found to be the least sensitive.

3.) The *in vitro* methods, used in this study proved to be appropriate for comparing the herbicide sensitivities of the various microbes. At the higher herbicide doses, which are causing a potential cell aggregation the "biomass assay test" could be suggested for further application.

B) Results of *in vivo* experiments:

1.) In the soil-incubation experiments the affecting periods are influencing on the abundance of studied microbes. At the short-time effect every rates of the chlorsulfuron (also the practical dose) was causing a significant inhibition of microorganisms. After the 3 months incubation, however a growth-stimulation was recorded at the practical herbicide rates, in accordance with the *in vitro* experiments. Adaptation and herbicide degradation-ability of microbes can influence on the microbial abundance both *in vitro* and *in vivo*.

2.) The herbicide and industrial waste water combinations modified the results of single herbicide experiments. Growth- inhibition effect of the combinations was found to be less particularly at the short-time experiments.

3.) Nitrogen-fixing bacteria proved to be the most sensitive ones at the chlorsulfuron and industrial waste water combinations, similar to *in vitro* experiments. Spore-forming bacteria have been also strongly inhibited resulting also their total elimination from the targeted soil. The growth-stimulation-effect (up till the 1000-fold doses) of chlorsulfuron shows a great adaptation and/or metabolisation ability of *Actinomycetes.* The different sensitivity patterns of the soil-microorganisms may lead to the quantitative and qualitative modification of the soil-microbial communities and the soil functions.

FURTHER PLANS AND SUGGESTIONS

According to the sustainable agricultural aspects such examinations of the soil fertility microorganisms can be useful and suggested by the applied methods of this study. The quantitative estimation of the microorganisms with the simple cultivation method is the most applicable, which offers a monitoring tool *in vitro*. Among the microorganisms particularly the nitrogen-fixing bacteria can be used as sensitive indicator-organisms in the various soils, treated by herbicides and other amendments. The nitrogen-fixation is the key function of the arable soils. The study of those microbes therefore can be strongly suggested for monitoring purposes. Other microbes, as the *Actinomycetes* and *Pseudomonas* can be also suggested for studying the herbicide effects of the soils. According to literary data those microbes are known herbicide-degraders, so they can be used also in the remediation reaugmentation of soils from the herbicide residues. Beside the

authentic strains, therefore it seems to be necessary to isolate and identify microorganisms, which are able to be degrade and metabolise the herbicides and other harmful chemicals, xenobiotics.

It can be also suggested to continue the microbial growth tests on the chlorsulfuron and other herbicides, industrial pollutants, wastes, including the waste-waters and sewage sludges in their combination with herbicides *in vitro* and also among the field conditions.

Before predicting the toxicity or the degradability of a certain herbicide (or other xenobiotics) it is recommended to consider both the short-term direct and the long-term indirect effects in the soil-plant environmental systems. Further long-term research activities are recommended, therefore on studying the interactions between microbes, herbicides and the various biotic and abiotic environmental factors.

References

Angerer I.P., Biró B., Köves-Péchy K. (1998): Indicator microbes of chlorsulfuron addition detected by a simplified soil dilution method. *Agrokémia és Talajtan*. 47: 297–305.

Angerer I.P., Ködöböcz L., Biró B. (2004): Sensitivity of some microbial groups against herbicide-wastewater combinations in model experiments. *Agrokémia, Talajtan.* 53: 331-343, (in Hungarian).

Angerer I.P., Rausch P., Biró B. (2006): Specificity of microbial sensitivities to chlorsulfuron *in vitro* and in soil-incubation experiment. *Acta Microbiological Immunologica Hungarica*, 53: 239–240.

Angerer I.P., Biró B. (2007): Dose- and time–dependent abundance of some soil-microbes at Chlorsulfuron herbicide application. *Cereal Research Communication,* 35 (2): 181-184.

Angerer I.P., Köves-Péchy K., Kecskés M., Biró B. (2007): Chlorsulfuron herbicide sensitivity of some microbial groups *in vitro* and in soil incubation model experiment. *Agrokémia, Talajtan.* 56: 147-160, (in Hungarian).

Biró B., Angerer I.P. (1997): Modified, soil-diluted selective method suggested for soil-monitoring and for environmental protection. In: *IX. Conference of Environmental Protection in Hungary,* Siófok. p. 287–293, (in Hungarian).

Reichart O. (2005): Evaluation of Scientific experiments in the microbiological practice. Budapest, pp. 97, (in Hungarian).

Stefanovits P. (1977): Soil- and Environmental protection. Mezőgazdasági Kiadó (Agricultural Editor), Budapest, pp. 243, (in Hungarian).

Szegi J. (1979): Soil-microbiological Manual., Mezőgazdasági Kiadó (Agricultural Editor), Budapest, pp. 310, (in Hungarian).

Thirunarayanan K., Robert L. Zimdahl R.L., Smika, D.E. (1985): Chlorsulfuron adsorption and degradation in soil. *Weed Science,* 33 (4): 558-563.

Vincent J.M. (1970): A manual for the practical study of nitrogen-fixing bacteria. Oxford Press. Sidney pp. 150.

LIST OF PUBLICATIONS

Publications on the topic of the doctoral (Ph.D) thesis

A./ Scientific articles in peer-rewieved journals:

ANGERER I.P., BIRÓ B., KÖVES-PÉCHY K., ANTON A., KISS E. (1998): Indicator microbes of chlorsulfuron addition detected by a simplified soil dilution method. *Agrokémia, Talajtan*, 47: 297–305, (in Hungarian).

ANGERER I.P., KÖDÖBÖCZ L., BIRÓ B. (2004): Sensitivity of some microbial groups against herbicide-wastewater combinations in model experiment. *Agrokémia, Talajtan*, 53: 331-343, (in Hungarian).

ANGERER I.P., RAUSCH P., BIRÓ B. (2006): Specificity of microbial sensitivities to chlorsulfuron *in vitro* and in soil-incubation experiment. *Acta Microbiologica Immunologica Hungarica*, 53: 239–240.

ANGERER I.P., KÖVES-PÉCHY K., KECSKÉS M., BIRÓ B. (2007): Chlorsulfuron herbicide sensitivity of some microbial groups *in vitro* and in soil incubation model experiment. *Agrokémia, Talajtan*, 56: 147-160.

ANGERER I.P., BIRÓ B. (2007): Dose- and time–dependent abundance of some soil-microbes at Chlorsulfuron herbicide application. *Cereal Research Communication*, 35: 181-184.

B./ Conference proceedings:

ANGERER I.P., BIRÓ B., KÖVES-PÉCHY K., KISS E. (1997): Chlorsulfuron-wastewater combinations affecting the abundance of some soil microbial groups. *Proc. 3rd Conference of Environmental Protection, Veszprém,* Hungary, p. 375-383, (in Hungarian).

BIRÓ B., **ANGERER I.P.** (1997): Modified soil-diluted selective method for monitoring the environmental status of soils. *Proc. 9th Hungarian Conference of Environmental Protection,* Siófok. p. 287–293, (in Hungarian).

ANGERER I.P., KÖDÖBÖCZ L., BIRÓ B. (2004): Sensitivity of microbes against herbicides and potentials of bioindications. *Proc. 13th Hungarian Conference of Environmental Protection*, Siófok, p. 168-175, (in Hungarian).

C./ Abstracts

KÖDÖBÖCZ L, FÜZY A., VILLÁNYI I., **ANGERER I.,** MORVAI B., BIRÓ B. (2004): Sewage sludge application of soils and results on rhizobiological parameters. *Abstracts of Soil Science Conference,* Kecskemét, p. 34, (in Hungarian).

ANGERER I.P., BIRÓ B. (2005): Model experiments to test sensitivity of microbial groups to combination of herbicide Chlorsulfuron and industrial wastewater. In: *Soil-Plant-Microbe Interactions, Fundamentals and Applications*. International Interdisciplinary Postgraduate Course, Uppsala, Sweden, p. 25.

Publications not attached directly to the Ph.D thesis

A./ Scientific publications

VILLÁNYI I., FÜZY A., **ANGERER I.**, BIRÓ B. (2006): Total catabolic enzyme activity of microbial communities. Fluorescein diacetate analysis. p. 441-442. In: *Understanding and modelling plant-soil interactions in the rhizosphere environment. Handbook of methods used in rhizosphere research.* Chapter 4.2. *Biochemistry* (ed. D.L. JONES). Swiss Federal Research Institute WSL, Birmensdorf.

BIRÓ B**, ANGERER I.P.**, VILLÁNYI I, KÖDÖBÖCZ L. (2005): Quality parameters of composted wastes by microbiological investigations. p. 21-26. *Proc. 13th Scientific Meeting of SzSzBTT of Hung. Acad. Sci.,* (Eds. KÓKAI S, MIZSUR B). Kapitális Print, Nyíregyháza, (in Hungarian).

BIRÓ B., **ANGERER I.P.,** KÖDÖBÖCZ L., BECZNER J. (2005): Food quality and safety of green pea by rhizobiological investigations in sewage sludge amended soils. p. 227. *Proc. of Rhizosphere 2004 Conference* (ed. by A. HARTMANN et al.). GSF Bericht; Germany

ANGERER I.P., BIRÓ B. (2005): Sensitivity of green-pea rhizosphere in sewage sludge-treated soils. *Proc. 19th National Conference on Environmental Protection*, Siófok, p. 173-179, (in Hungarian).

BIRÓ B., VILLÁNYI I., FÜZY A., KÖDÖBÖCZ L., **ANGERER I.**, MAKÁDI M., ANTON A., MONORI I. (2008): Microbial activity and potential control at the utilization of organic wastes in the agriculture. *Talajvédelem* (Suppl.), p. 152-161, (in Hungarian).

B./ Other publications

ANGERER I.P. (2002): Programs of Environmental Protection of Dunaújváros MJV: 1st audited Version, Duna-Print Comp. Dunaújváros. pp. 189, (in Hungarian).

ANGERER I.P., TÓTH L., MÉSZÁROS R. (2004): Programs of Environmental Protection of Dunaújváros MJV: 2nd audited Version, Text Print Comp. Dunaújváros. pp. 187, (in Hungarian).,

ANGERER I.P. (2005): Experience on the applicability of environmental protection program of Dunaújváros. Publications of College *Dunaújváros*, 26: 493-501, (in Hungarian).

ANGERER I.P., TÓTH L., TÓTH T., SZÁNTÓ K. (2008) Environmental Status of Dunaújváros MJV. TEXT Print Comp.. Dunaújváros, pp. 76, (in Hungarian).

GÁL N., **ANGERER I.P.,** TÓTH L., TÓTH T., CZEGLÉDI A. (2008): „Whom environment?" – the environmental site of Baracs street. TEXT Print Comp., Dunaújváros, pp. 18, (in Hungarian).