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Diploma thesis

The influence of plant growth on pesticide disappearance from the soil

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Declaration

I declare that my thesis "Influence of plant growth on pesticide disappearance from soil" I worked out separately under the supervisor of the thesis and using literature and other information sources that are cited in the work and listed in the bibliography at the end of work. As author of the thesis further declare that I am related to its creation did not infringe the copyright of third parties.

In Prague on April 13, 2012 _____

Acknowledgements

I want to acknowledge Martin Kocarek, Ph.D for generous help and advice on both experimental and laboratorial analysis, to Department of Soil Science and Soil protection of Czech University of Life Sciences Prague for giving opportunity for performing experiment and supporting all materials, Ministry of Education, Youth and Sport of Czech Republic for financial support during entire study period.

Summary

The influence of growth on disappearance of chlorotoluron from the soil was studied. Chemical classes and some important physicochemical properties of chlorotoluron, which are important for degradation process, are briefly described. Consequently, the fate of chlorotoluron in soil, air and water, especially importance of microbial degradation, involving factors such as soil type, moisture, organic matter content, pH, microbial diversity, are considered. In addition, the soil properties which plays critical role in herbicide behavior are described. The bucket experiment was carried out in the greenhouse, and chlorotoluron concentration has determined by means of HPLC 1, 7, 14, 24, 35, 49, 74, 97 and 200 days after application. Calculated half-lives for chlorotoluron ranged between 99.7 days to 137.2 days. Selected growths and irrigation influence on disappearance of chlorotoluron from the soil are statistically analyzed. The correlation between concentrations of chlorotoluron vs. time, growths and irrigation are tested. Discussions based on the results, and conclusions are made at the later part of the thesis. At the end, used references and appendices are available.

Keywords: Chlorotoluron; Microbial degradation; Half-life; Persistence

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1. INTRODUCTION

World population increased rapidly in last 6 decades up to 7 billion. These followed by intensive agriculture development all over the world to supply people with food. Application of pesticide to arable land is one of the important practices for controlling the weed growth in modern agriculture. While it has primary benefits: Improving yields, its quality, appearance, safety and extending shelf-life by reducing pest, epidemics and controlling invasive species, also has secondary benefits in different levels. For example: food safety and security, wider range of viable crops, improving quality of life, increasing life expectancy and labour freed for farming communities. Furthermore, in national level it influences agricultural economy, export revenues, nutrition and health, human productivity, reducing soil erosion and moisture loss. Globally, diverse produce, less pressure on uncropped land, less green house gas which has negative impact on global warming, assured safe food supply and conservation of biodiversity as well.

Like many technological developments that improve our life, pesticide may pose some risk for human and environment if they are not used with due care and consideration. Contamination of the environment caused by pesticide application is a worldwide problem because of bad agricultural practice and persistence of chemical itself.

While the pesticide has consequences of effects – direct gains expected from their use. Soil is the main recipient of pesticides used for plant protection, but the pesticides were also detected in surface and ground water, water sediments, air and in the final agricultural products. Before pesticide reach the soil, the active substance may reach undergo photodecomposition, it may be transformed by air and adsorbed by plant. Once it enters the soil, it is subject to various transformation and transformation processes. The knowledge about the pesticide, its behaviours are important for the pesticide fate in soil and for the environmental protection.

Determining pesticide half-life time and having knowledge about its environmental fate, its contamination level, ecotoxicity, and human and animal health risk are the most important issue to be considered, while we have no way to avoid using them. It will be one-sided if pesticide should not be used for agricultural practice. On the other hand it has much more benefits, mentioned above, are the main driving force for using pesticide over several decades.

2. AIM AND OBJECTIVE

The aim of this study was to calculate the half - life of herbicide chlorotoluron and to evaluate the effect of different types of plant species on chlorotoluron disappearance in the soil. However chlorotoluron was studied a lot, this study was specific for chlorotoluron disappearance from the soil by influence of different plants, under green house condition.

Following hypothesis was raised:

1. H_0-The plant variants have influence on degradation of chlorotoluron
   H_1- The plant variants have no influence on degradation of chlorotoluron
2. H_0- The irrigated water have influence on degradation rate (half - life of herbicide)
   H_1- The irrigated water no influence on degradation rate (half - life of herbicide)

3. LITERATURE REVIEW

3.1. Chemical classes and physico-chemical properties of chlorotoluron

Chlorotoluron (Fig. 1) is soil-acting, synthetic urea herbicide which is used to control broadleaf and annual grass weeds. It has IUPAC name of 3-(3-Chloro-4-methylphenyl)-1, 1-dimethylurea, molecular formula $C_{10}H_{13}ClN_2O$ and molecular weight of 212.7 g mol^{-1}. It acts as an inhibitor of photosynthetic electron transport of the plants and selective, non-systemic absorbed by roots and foliage. It inhibits not only target weed species but also depresses the biochemistry of agricultural plants (Song et al., 2007).

Chlorotoluron was firstly introduced (registered or discovered) in 1994, in Spain and approved for use in following European countries: Belgium, Bulgaria, Czech Republic, Germany, Spain, France, Hungary, Italy, Portugal, Romania, Slovenia, Slovakia and United Kingdom.

Synonyms: N-(3-Chloro-4-methylphenyl)-N',N'- dimethyl urea; Dicuran; 3-(3-chloro-p-tolyl)-1,1-dimethylurea; Chlortokem; N'-(3-chloro-4-methylphenyl)-N,N-dimethylurea; N-(3-Chloro-4-methylphenyl)-N',N'-dimethylurea; Tolurex; 3-(3-chloro-4-methylphenyl)-1,1-dimethylurea and some other trade names such as Syncuran 80DP, Tolerate, Alpha chlorotoluron 500, Tolugan and Tolugan extra.

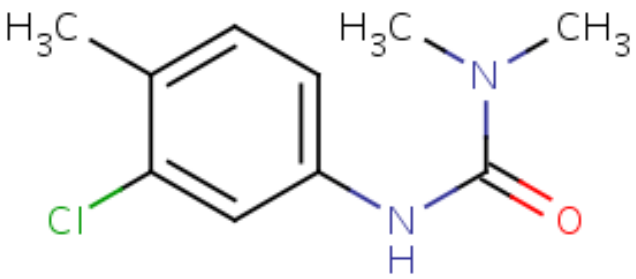


Fig. 1: Molecular structure of chlorotoluron <http://www.brenda-enzymes.org/php/ligand_flatfile.php4?brenda_ligand_id=84446>

In biological media there is competition between oxidation of the ring methyl and N-demethylation processes, and the balance between rates of these two pathways provides the basis of selectivity of herbicidal action for the compound. Chlorotoluron is stable to heat and UV light and slowly hydrolysed by strong acid and alkalis (Directive

91/414/EEC). Hydrolytic stability and photostability in water are quit long, has half-life of >1 year. The identity, physical and chemical properties of chlorotoluron is shown in Appendix I. The most important properties of chlorotoluron for its environmental fate are described briefly below.

3.1.1. Water solubility

Solubility, "*The mass of given substance/solute that can dissolve in a given volume of water/organic solvent*", which is chemical-specific characteristic and some are pH sensitive (Pesticide Properties Data Base (Directive 1107/2009 (repealing 91/414))). Chlorotoluron has low (see Appendix II) water solubility, 74 mg l^{-1} at 20^0C, and less soluble compared to organic solvents which have solubility of several g l^{-1}, exception of n-Hexane which has solubility of 60 mg l^{-1} at 20^0C. Solubility measure refers to how readily the herbicide dissolves in the water. This characteristic determines the extent to which an herbicide is in the solution phase or the soil phase. Water solubility plays a major role in determining the fate of herbicide in water, soil and air. An herbicide that is water soluble will not be retained by the soil (Miller and Westra. Herbicide behavior in soil. Crop series, No 0.562 <http://cospl.coalliance.org/fez/eserv/co:5589/ucsu2062205621998internet.pdf>).

3.1.2. Octanol-water partition coefficient (K_{ow})

The partition coefficient is a ratio of concentrations of un-ionized compound between the two solutions. The logarithm of the ratio of the concentrations of the un-ionized solute in the solvents is called log *P*: The log *P* value is also known as a measure of lipophilicity. A classification of pesticides as fat-soluble has been proposed for compounds with a log $P_{ow} > 4$ (Alan, 1993). The log P_{ow} may be used in estimating the environmental behaviour of pesticides. log P_{ow} value of chlorotoluron is 2.5 ± 0.1 at 25^0C, pH=7 condition (WHO, 2003).

3.1.3. Vapour pressure

The vapour pressure is chemical-specific property, defined as the partial pressure of a chemical, in the gas phase in equilibrium with the pure solid or liquid chemical. Vapour pressure is very pH dependent. This parameter governs the distribution between liquid and gas phase or between solid and gas phase. It is expressed in both Pa and mmHg unit, measured at 20-25^0C. Vapour pressure of chlorotoluron is 5 x 10^{-6} Pa at 25^oC.

3.1.4. Henry's Law constant

Henry's Law constant, denoted H or K_H is a partition coefficient defined as the ratio of chemical's concentration in air to its concentration in water at equilibrium. This parameter is important for tendency of chemical volatilization from water solution into air. It is generally considered that compounds with H value $>10^{-5}$ Pa m^3 mol^{-1} have little tendency to volatilize. Henry's Law constant for chlorotoluron is not determined due to very low vapour pressure (WHO, 2003). Volatility of substance at given temperature is higher when the vapour pressure is high. Which means that volatilization of chlorotoluron itself cannot be take place without water. Weak vapour pressure does not always indicate a negligible volatilization. Volatilization of chlorotoluron from soil and plant surface has been determined less than 2.1% of the total concentration according to the Atkinson calculation (WHO, 2003).

3.1.5. Soil sorption coefficient (K_{oc})

Partition coefficient of organic carbon (K_{oc}), which is chemical-specific, describes the ratio between chemicals and adsorption on carbon and its concentration in water, is one of the factors that influence the herbicide fate. A first characterization is the measurement of the simple "sorption" coefficient, K_d, defined as the ratio of the concentration of the chemical adsorbed on soil to the concentration of pesticide in the soil solution. K_d value has good correlation with SOM indicates that the principle adsorption mechanism involves interaction between the pesticide and the organic matter component of the soil. Therefore, the adsorption coefficient is normalized to take into account the different SOM or organic carbon content.

K_{oc} values are useful in predicting the mobility of organic soil contaminants; higher K_{oc} value correlate to less mobile organic chemicals while lower K_{oc} value correlates to more mobile organic chemicals <https://fortress.wa.gov/ecy/clarc/FocusSheets/Physical&ChemicalParameters.htm>. Significant linear correlation between log $_{Kow}$ and log $_{Koc}$ was observed for phenylurea herbicides (Jianbo and Chuanfan, 1995). As K_{oc} value increases the adsorption (Kodesova et al., 2011) of chlorotoluron, bioaccumulation of chemical increases and leaching and biodegradation decreases. In addition, the octanol-water partition coefficient (K_{ow}) is needed to calculate K_{oc}. Pesticides with K_{oc} values below 50 are considered to be high mobile; values of 150-500 signify moderate mobile, and above 200, slightly mobile compounds.

3.1.6. Persistence

Persistence of herbicide is "*The longevity of herbicide molecule is normally expressed in terms of half-life (DT_{50}), as determined under normal condition in the region where it is used*". This measure also can be length of residual weed control and is generally expressed in some unit of time (days, weeks or years) (Miller.P., and Westra.P., Herbicide behavior in soil. Crop series, No 0.562 <http://cospl.coalliance.org/fez/eserv/co:5589/ucsu2062205621998internet.pdf>.

The persistence of chlorotoluron differs from moderate to very long (see Appendix II for classification) depending on half-life, that found from studies in different conditions. Persistence is not fixed property of the chemical but is influenced by factors such as soil type and the weather conditions after application. Thus, persistence of the chemical is specific to one particular location and season since the soil type and weather conditions vary from site to site and year to year (Hance, 1980). Also persistence of several pesticides may be changed when used in combination of other pesticide or after repeated applications (Vischetti et al., 2008).

Tab. 1: The factors influencing the pesticide persistence in the soil (Manuel et al., 2008)

	Factors
Pesticide	Chemical nature, volatility, solubility, formulation, concentration, application, method used, time of year and day, frequency of application, amount of pesticide
Soil	Soil type, texture, clay content, structure, compaction, organic matter and humus content, soil moisture, pH, mineral ion content
Site	Elevation, slope, aspect, geographical location, plant cover (species, density, distribution, history at site), microbial populations (species, density, distribution, history at site), use of " fertilizer", lime, mulches and green manures, use of other pesticides and herbicides, tillage, cultivation, drainage, irrigation (type, depth, amount, amount, timing, frequency), fire, adjacent environment, presence of pollutant,
Climate	Wind, air movement, temperature, solar radiation, rainfall, relative humidity, evaporation
Experimental variables	Plot size, arrangement, number of replicates, frequency of sampling, sample size and shape, techniques for measuring variables.

Performing experiment for determining persistence in the lab condition has advantage of well controlled conditions of temperature, light and humidity, but is far from real conditions, because of the lack of vegetation and intense microbial activity, filed experiments are certainly more reliable because they are made under real usage conditions.

Half-life, valuable tool in comparing rate of herbicide degradation, is the time required for the herbicide concentration under conditions to decline to 50 % of the amount at application. In many cases herbicide shows "half-life" behavior, in which subsequent concentration continue to decline by 50 % in the same amount of time. In such cases several DT_{90} whose concentration declines under defined condition to 90 % of the amount at application, is used (Pesticide Properties Data Base (University of Hertfordshire-EC Directive 1107/2009 (repealing 91/414))). Half-life of chemical determines its persistence.

3.1.7. Mobility

The mobility of the pesticide in soil is relevant to their leaching, volatilization and bioavailability to flora and soil. This mobility is largely dominated by sorption processes following a variety of mechanisms. These processes depend on the lipophilicity of the pesticide, the soil mineralogy and inorganic matter content, and especially the soil humidity. In general, it has been stated that decreasing water content increases adsorption and reduces the mobility, increasing temperature reduces the adsorption and increase mobility, increasing clay mineral content and organic matter content increase sorption and reduce the mobility, and that plant cover increases metabolism and reduces leaching.

However, soil is a complex biological and chemical medium, the kinetics of adsorption and desorption might effect rates of loss by controlling availability for degradation, the activities of soil microorganisms may vary with time depending on the availability of nutrients and other energy sources, and there may by competing reaction sequences within the degradation process (Hance, 1980).

3.2. Chlorotoluron degradation in soil

In chemistry degradation is defined as "*The process which is the reduction of chemical compound to one or less complex, as by splitting off one or more groups*". As explained in mass conservation law nothing will disappear but changed into metabolites by loosing and combining functional groups and split into 2 and more compounds or

changes its state. The energy is also included in this term. At the end of the experimental work either entire herbicide molecule or its degradation products and derivatives of chlorotoluron molecule can be found. Some part of the herbicide may adsorb to surface of soil particle in lack of water and soil organic matter which can change chlorotoluron fate in the soil. End products of herbicides (organic chemical) are H_2O and CO_2.

Chlorotoluron is one of the phenylurea herbicides which can be encountered at concentrations reaching several $\mu g\ l^{-1}$ in the water (Carabias-Martinez et al., 2002; Field at al., 2003). About chlorotoluron degradation together with influencing factors in special conditions at both field and laboratorial conditions, was studied and published.

Chlorotoluron degradation takes place 1) in soil, 2) in water and 3) in the atmosphere.

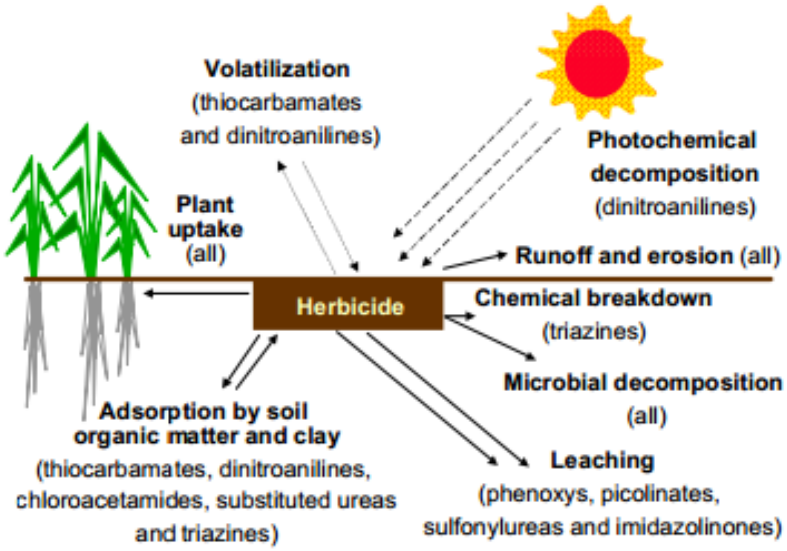


Fig.2: Processes that make herbicides inactive <http://cospl.coalliance.org/fez/eserv/co:5589/ucsu 2062205621998internet.pdf>.

Chlorotoluron degradation can be divided into 3 main divisions according to its way of degradation: 1) biological (microbial), 2) chemical and 3) physical.

The most important process which involves to degradation of pesticide is carried out by microorganisms (biological degradation) in the soil profile. In addition, oxidation - reduction potential and hydrolysis (chemical degradation), photodecomposition (physical degradation) are also play critical roles.

All these degradation ways, factors influencing degradation, physical and chemical properties of the soil, which are important for degradation cannot express fate of herbicide alone, they are related to each other in very complicated way.

The soil is the main recipient of the herbicide where it can be applied both pre - and post - emergently, fate of the herbicide takes place in the water phase of the soil. Soil structure, its porous system and hydraulic properties are the most important parameters of the soil water and its ability to take water from surrounding. These parameters influenced by many other soil factors including organic matter content, soil water regime, mineralogical composition of parent rock material, transport processes in the soil profile, climate, plant root, soil organism and management practices (Kodesova et al., 2009). Many of these factors involves in herbicide degradation, adsorption and transportation in the soil.

The degradation rate of pesticide in the soil may effected by factors for example soil structure and type, organic matter content, porosity, hydraulic conductivity, adsorption by the soil particles where the herbicide was applicated reported by several authors (Kordel et al., 1996; Groen, 1997; Lluch, 1997; Beulke at al., 2004; Kodesova et al., 2005). Half-life is not only affected by factors which mentioned above but also soil moisture content, temperature, canopy type, varieties of the microorganism species in the soil and their content at that particular time of studying.

3.2.1. Microbial degradation

The diversity of species of microorganisms in soil is great. Interaction of abiotic and biotic factors forms dynamic equilibrium of microbial population which can modify environmental conditions. For example, in the region of plant roots, the rhizosphere, the numbers, types and metabolic activity of microorganisms and the physicochemical soil conditions are different from those in soil outside the rhizoshpere (Hance, 1980). Thus, microorganisms in the soil influence fate of pesticide in the environment and their degradation rate (Aislabie and Lloyd Jones, 1995) to prevent accumulation of chemical in the environment. Microbial degradation rate of pesticide could influenced by many environmental factor such as temperature, pH, availability of nutrient, electron acceptor, presence of competitors and predators, which influence the microorganisms growth (Chapman and Harris, 1990).

The soil pH has great influence on soil microorganism community including fungus, ratio between them and the distribution of functional and taxonomic groups (Baath and Anderson, 2003). It showed different features of metabolism of pesticide depending on the pH value in study of atrazine (Huout et al., 2000). In contrast, in some studies the pH value had no significant influence to pesticide (isoproturon) degradation (Gary et al., 2007). The degradation of herbicide depends on the soil and chemical properties and microorganism ability to degrade particular pesticide. In addition, pH plays critical role for sorption of the pesticide to soil organic matter thus its bioavailability decreases.

Degradation rate of herbicide (isoproturon) declines progressively down to the soil profile was associated with the starting population size of catabolic organisms or the number of catabolic organism proliferating following 100 % degradation and with an increase in the length of lag phase prior to exponential degradation, suggesting the time required for adaptation within community controlled degradation rates (Gary et al., 2007). Gary et al., (2007) demonstrate that sorption did not change with soil depth, despite a substantial reduction in organic matter and found no significant change with soil depth in the maximum rate of degradation during the exponential degradation phase, suggesting that soil depth had no significant effect on pesticide bioavailability. These findings indicate that bioavailability was not a factor influencing adaptation of organisms to degrade the pesticide or the growth and dynamic of adapted organism (Gary et al., 2007). Increased pesticide persistence in autoclaved soil support the observation that biodegradation is very important to the dissipation of pesticide in the soil (Tariq et al., 2006).

One more factor that increases the rate of microbial degradation of pesticide in soil is one or more previous application of the same pesticide or another pesticide with a similar structure. This phenomenon is known as accelerated or enhanced degradation which means that microorganisms get adapted to degrade pesticide. It followed by repeated application of pesticide because of insufficient concentration of pesticide to supply crop protection for the length of time required (Ziv et al., 2007). Accelerated degradation of pesticide can occur after first application. Vanhala et al., (2008) reported that microorganisms had no adaptation of SOM decomposition to the prevailing conditions leads to different temperature vs. decomposition curves for different soils. One reason for this could by an adaptation of microbes to the surrounding climate conditions, and another reason could be an adaptation to different carbon substrates available in the soils.

The degradation rate and extent of microbial growth during degradation is also influenced by the structure of pesticide itself. Pesticides with simple structure which is similar to naturally occurring substances are tends degrade quickly than the pesticide with more complex structures. In contrast, pesticides with a structure that is different from naturally occurring substances (xenobiotics) often degraded slowly since the microorganisms do not posses suitable degradation genes (Ziv et al., 2007). Phenylurea herbicides including diuron, linuron, monuron, metobromuron and chlorotoluron which have N-methoxy-N-methyl and N, N-dimethyl side chains are degraded by several soil fungi. In study, chlorotoluron degradation by the fungi, *Mortierella* sp.Gr4 strain, has ability to degrade group of phenylurea herbicides, chlortoluron gave another metabolite other than the N-demethylation but also hydroxylation of isopropyl ring substituent, is recorded (Nora Badawi et al., 2009).

The pesticide molecule which cannot be degraded completely by influence of the microorganism could be transformed to another form which can be more toxic to living organism in the soil. The biotransformation of phenylurea herbicide including chlorotoluron by *Arthrobacter* sp. N2 studied by Tixier et al., (2002) showed complete degradation in the bacterial medium but no balance between loss of herbicide and formation of anilines was observed. The highest depletion of parent molecule was 26%, which has likelihood of chemical to transformed to metabolites which is not determinable by analytical method (inappropriate analytical method to determine those chemicals) or followed by mineralization process (Tixier et al., 2002).

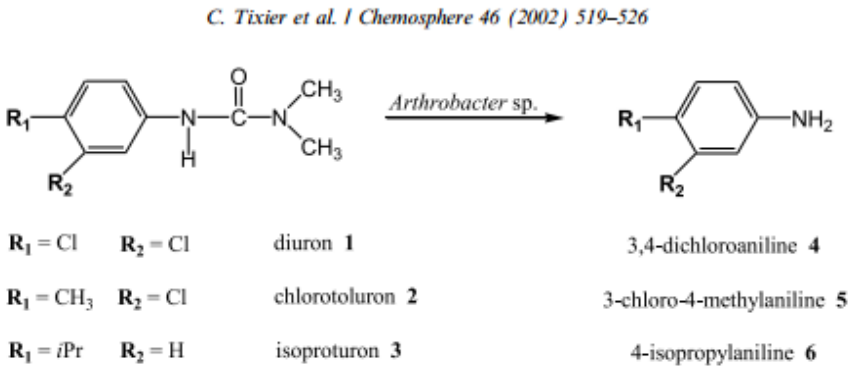


Fig. 3: General scheme for biotransformation of *Arthrobacter* sp. N2 of three phenylurea herbicides: diuron (1), chlorotoluron (2) and isoproturon (3). (Tixier et al., 2002)

Microorganism species have variable ability to degrade herbicide molecule. Castillo et al., (2006) studied diuron (urea herbicide) degrading activity of 17 streptomycete strains isolated from soil. The result showed that all *Streptomyces* sp. which differs by their phenotypic characteristic had biodegradation activity up to 50 -95 % in laboratory condition (Castillo et al., 2006). Studying activity of biodegradation of microorganism species for specific pesticide will be helpful to bioremediation of soil which contaminated with pesticide.

Usage of biomix such as biobed (biological system used to reduce point source water contamination by pesticide) can be one of the possible protections to water bodies against pesticide contamination and considered a suitable medium for treating pesticide in biological systems. Because it was accelerated the pesticide (metalaxyl and chlorpyrifos) degradation and microorganism community in biobed arrive to new metabolic equilibrium within 20-40 days after application of pesticide (Vischetti et al., 2008).

In order to estimate or predict persistence of herbicide in different situations, affect of soil type and environmental factors which involves to degradation rate of herbicide must be known. Degradation of some compounds is characterized by an initial lag-period during which little or no change in concentration usually have rapid degradation and appears linear in time. But detailed analysis may show that it follows first-order kinetics. On the other hand, degradation rate of compounds which has no lag-period are more or less proportional to concentration of pesticides, so that the result can be interpreted using first-order kinetics (Hance, 1980). The equations for first-order reaction and half-life are:

$$\mathbf{C = C_0 * e^{(-kt)}} \qquad \mathbf{(1)}$$

where: **C** – concentration of chlorotoluron after time t, $\mu g\ g^{-1}$

$\mathbf{C_0}$- initial concentration of chlorotoluron, $\mu g\ g^{-1}$

k - rate constant for chemical disappearance

t - time, days

A plot of the logarithm of concentration against time gives a straight line with proportional to the rate constant.

$$DT_{50} = \ln 2 / k \qquad (2)$$

where: DT_{50} – half-life, days

k - rate constant for chemical disappearance

The half-life in first-order reaction is independent of initial concentration but this is not so for other order of reaction.

Beside microbiological degradation, oxidation and reduction potential of the soil, hydrolysis of herbicide in the soil and photodecomposition as a physical degradation. Hydrolysis has major degradation influence than microbial degradation in sandy soil (Damia and Marie, 1997).

3.2.2. Soil properties effect herbicide behaviour

The behaviour of pesticide in governed by a variety of complex dynamic physical, chemical and biological processes shown in the Tab. 2 (Manuel et al., 2008). These processes directly control the transport of pesticides within the soil and their transfer from soil to water, air or food. The relative importance of these processes varies with the chemical nature of the pesticide and the properties of the soil (Manuel et al., 2008). But degradation of pesticide is governed by biotic and abiotic factors, and can follow complex pathways involving a variety of interactions among microorganisms, soil constituents and pesticide itself. The most important soil properties involving to behaviour of pesticide are briefly described below.

3.2.2.1. Organic matter

On the basis of organic matter content, soils are characterized as mineral and organic. Mineral soils form most of the world's cultivated land and may contain from a trace to 30 % organic matter. Organic soils are naturally rich in organic matter principally for climatic reasons (FAO soils bulletin 80). The total amount of organic matter in the soil is influenced by – soil texture, environmental temperature, soil moisture, aeration, clay mineral and biological activity, topography, salinity and acidity of the soil and by the quantity of annual of plant and animal residues to the ecosystem (FAO soils bulletin 80). Organic matter in soil modified by time in following order: organic residues (plant,

animal, microorganism) → Nonhumic substances (easily degradable and simple structured substances) → humic substances (fulvic acid, humic acid, humins). But soil organic matter can be partitioned conveniently into different fractions, do not represent static end products. Instead, the amounts present reflect a dynamic equilibrium.

While humic substances are less mobile and less soluble, they are more stable and tend to have greater stability and more resistant to microbial degradation, soluble organic matters, which are relatively mobile have inverse characteristics. Organic matter has great importance to pesticide bioavailability, mobility, adsorption, leaching and degradation processes. Up to 6% of organic compost has trend to decrease the mobility but further increase in the organic compost concentration has noticeable (7%) and slightly (8%) increase in pesticide mobility (Kodesova et al., 2011). Soil, which has high amount of organic matter, has high sorption and water holding capacity.

Being rich in organic matter, influences on physical features of the soil. Organic matter together with clay particles which are main cementing agent can improve the soil physical and chemical properties such as soil aggregate, compaction of soil, hydraulic conductivity ((Zebarth et al., 1999), (Franzluebbers et al., 2002), (Pagliai et al., 2004), (Garcia-Orenes et al., 2005), (Tejada and Gonzalez, 2006), (Kodesova et al., 2008), (Tejada et al., 2009) and (Hemmat et al., 2010) and decrease pesticide mobility in soil (Briceno et al., 2007).

Clay content, especially mineral fraction of clay influences the adsorption of pesticides. Organic matter has stronger effect, but the adsorption pesticide on to clay is important because must soil contain much more clay than organic matter (Damia and Marie, 1997).

3.2.2.2. Dissolved organic matter (DOM)

DOM, the most mobile soil organic fraction, which consists of small organic acids and macromolecules such as enzymes, aminosugars and polypheneol and organic compounds plays important role in many chemical and biological processes in the soil. Through physical and chemical binding it DOM is involved in co-transport of metals and some of xenobiotics (e.i pesticides). Also it decreases bioavailability of chemicals. DOM influences chlorotoluron action in the wheat metabolism depending on the DOM type. DOM also alleviates chlorotoluron toxicity and prevented cellular toxic effects by

reducing chlorotoluron accumulation in wheat (Song et al., 2010). But in absence of DOM adsorption of chlorotoluron was greater (Yang et al., 2005). In contract to organic compost addition (Kodesova et al., 2011), addition of dissolved organic carbon (Song et al., 2008) was increasing the mobility of chlorotoluron and its concentration of leachate in columns. DOMs (which is extracted from sludge and straw) have great positive influence of sorption of chlorotoluron and decreases its percolation to groundwater (Song et al., 2008) which is easily contaminated by organic pollutants applied on soil and followed by leaching or infiltration.

The soluble organic matter in the soil had no effect on sorption characteristics of some pesticides (atrazine, isoproturon and paraquat), is reported in Spark and Swift.2002. In the same study 2, 4-D sorption characteristic had affected by soluble organic matter due to the interaction of 2, 4-D with the soluble fraction of the organic matter. Also DOM had a critical role for increase in desorption of chlorotoluron from soil (Song et al., 2008). Not only organic carbon content but also other soil properties plays important role in chlorotoluron adsorption (Kodesova et al., 2011).

3.2.2.3. Temperature

The temperature has indirect effect on degradation rate of the herbicide in the soil. Temperature, varies depending on environmental condition, has influence on microbiological reaction and chemical interaction in the soil profile. Its sensitivity of decomposition (process important for organic matter development) decreases with increasing temperature (e.i. Vanhal et al., 2008). They also found that soil organic matter mineralization was equally dependent on temperature in all type of soils they studied. This study result might raise assumption that microbial activity which is responsible for mineralization of soil organic matter is dependent from temperature.

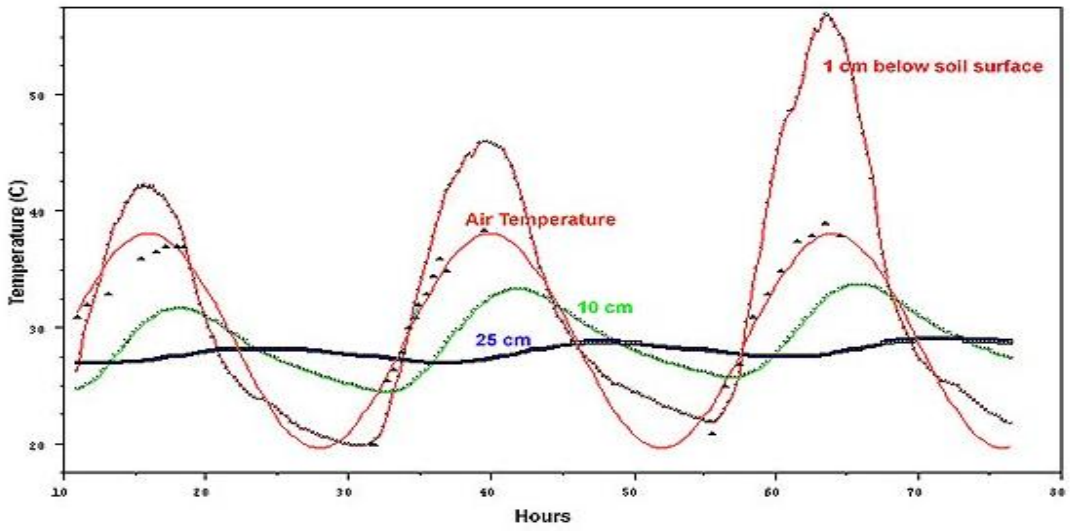


Fig. 4: Fluctuation of soil temperature at different depth vs. time. (http://www.usyd.edu.au/agric/ACSS/sphysic/temperature.html).

The top soil, which has high amount of organic matter and herbicide concentration, has greater fluctuation of temperature. The highest concentration of chlorotoluron found in top 2 cm, majority of it found in top 4 cm of the soil (Kocarek et al., 2010). This layer of the soil might have influenced by temperature rather than the chlorotoluron transported to deep layer of the soil. Higher temperature increase the degradation phenomenon in the soil was observed in laboratory incubation study by Tariq et al., (2006).

3.2.2.4. Cation exchange capacity (CEC)

Cation exchange capacity plays significant role to the mobility of some pesticides. This capacity has been correlated negatively with the movement of pesticide and positively with adsorption (Damia and Marie, 1997). CEC indicated increasing trend with increasing compost fraction (Kodesova et al., 2011). Also clay content in the soil contributes significantly to CEC (Damia and Marie, 1997).

3.2.2.5. Soil pH

Soil pH, acidity, affects the chemical properties of many pesticides. As soil pH decreases, pesticide bind more to the clay in the soil and filtered out of the percolating water. Also, pesticides in low soil pH value tend to be less soluble in the water. Soil pH can be determined in water and KCl, donated as pH_{H2O} and pH $_{KCl}$, respectively. While pH_{H2O} has decreasing trend with increasing of compost fraction, pH $_{KCl}$ has increasing

trend (Kodesova et al., 2011). The influence of pH on microorganisms in the soil is described in 3.2.1.

3.2.2.6. Adsorption

"*Adsorption is a physical process which adhere chemicals (concerning atoms, ions, biomolecules or molecules of gas, liquid or dissolved solids) to surface"*. Soil adsorption is measured by K_{oc} value. Higher values (greater than 1000) indicate a pesticide that is very strongly attached to soil and is less likely to move unless soil erosion occurs. Lower values (less than 300-500) indicate pesticides that tend to move with water and have the potential to leach or move with surface runoff. Adsorption depends on 1) the molecular properties of the solute - electronic structure, molecular volume, water solubility, 2) soil constituents - soil mineral, soil organic matter, organo-mineral association and 3) characteristic of the medium in which adsorption occurs - temperature, composition of solute, soil water content (Hance, 1980).

Adsorption - desorption of the chlorotoluron is associated with mobility, degradation, volatilization, bioaccumulation and finally fate of pesticide (Song at al., 2008) moreover, distribution of herbicide in the soil (Hance, 1980). Once, chlorotoluron adheres to surface of the soil particle then its bioavailability and mobility decreases depending on the strength of adhesion. Adsorption and organic carbon content are positively correlated in the many studies. Freudlich's adsorption coefficient (K_F) and soil organic carbon content (Hiller et al., 2008), are positively correlated and it has linear relationship with soil organic carbon content (Meyer-Windel et al., 1997). For chlorotoluron, this coefficient has significant correlation with CEC, Basic cation saturation (BCS), organic matter and $CaCO_3$, but no correlation to pH_{KCl}, pH_{H2O}, exchangeable acidity (EA), hydrolytic acidity (HA), sorption complex saturation (SCS), salinity and bulk density of the soil (Kodesova et al., 2011).

Adsorption and desorption isotherm of the chlorotoluron on the soil in presence of DOMs is examined and parameters are fitted to Freudlich equation by Song et al., (2008). The study showed that DOM leads to reduction in chlorotoluron sorption to the soil.

Study of chlorotoluron adsorption and desorption hysteresis in river sediments showed, it has higher sorption and lower desorption than the other phenylurea herbicides (especially isoproturon). Thus it has higher K_{oc} (organic carbon normalized sorption

coefficient) value depending on probably its stronger H-bonding interaction of the herbicide molecules with the sorbents. Logically, as desorption hysteresis increases sorbed amount increases. It tends to have higher K_{oc} value in the upstream river sediments than the downstream one. Moreover, it exhibited lower desorption potential than the isoproturon where in upstream river sediments (Chefetz et al., 2004)

There are two main factors controlling the sorption of chemical: the concentration of chemical in the soil and chemical structure of the soil or sedimentary organic matter (SOM) (Chefetz et al., 2004).

3.3. Chlorotoluron fate in the air

Fate of chlorotoluron in the air discriminated into two discrete processes: volatility and photodegradation. Without water vapour in the air the fate of chlorotoluron in the air is almost negligible.

Direct photolysis of chlorotoluron in the air is 2 to 10 hours (WHO, 2003). The photolysis of chlorotoluron in acetonitrile, hexane and aqueous solutions buffered by pH 4, 7 and 9 has investigated by Millet et al., (1998). The result showed that chlorotoluron was degradable by $\lambda<240$ nm, but photostable when $\lambda>240$ nm. In aqueous solution of chlorotoluron, mail product was found 3-(3-hydroxy-4-methyl-phenyl)-1,1-dimethylurea, while the quantum yields for photodegradation were ≈0.07 in aqueous and buffered solutions, ≈0.035 in acetonitrile, and 0.70 in hexane (Burrows et al., 2002).

Photolytic degradation of chlorotoluron at $\lambda=304$ nm, at 20-25^{o}C during 20 days, is reported in WHO report (2003). Javier Benitez et al., (2006) studied photodegradation of phenylurea herbicides including chlorotoluron and found out that only UV radiation is the driving force for degradation process. No other additives to water and water types (ultra-pure water, commercial mineral water, groundwater and lake water) gave any adverse results to their study, has been reported (Javier Benitez et al., 2006). Cleavage of chlorotoluron ring (transformation process) by influence of irradiation in air saturated solution 1.6×10^{-4} M, at $\lambda= 254$ nm was observed (Tixier et al., 2000). But demethylation and oxidation were the ways transformation of chlorotoluron when it was irradated on sand (no water). These transformation processes described by presence halogen atom on ring has significant influence on photochemical behaviour of chlorotoluron in the water (Tixier et al., 2000).

Chlorotoluron concentration in the air can be explained by both transportation process (volatility) and degradation process (photodegradation).

3.4. Chlorotoluron degradation in water

Chlorotoluron can be degraded abiotically and biologically in the water. Abiotic degradation of chlorotoluron can be divided into hydrolytic and photolytic. According to review report for active substance of chlorotoluron (WHO, 2003), it degrades more quickly in the hot temperature (above 50^{o}C) and tend to have high (more than 200d) persistence in lower (below 30^{o}C) temperature due to hydrolytic degradation process. Also Javier Benitez at al., (2006) found similar features of phenylurea pesticide degradation associated to temperature.

pH value of water, changes degradation rate 2.4 to 2.7 times higher at temperature above 50^{o}C. But in lower temperature (at 20^{o}C) pH (value 2 to 9 was studied) has no significant influence on chlorotoluron photodegradation (Javier Benitez et al., 2006). It is still not clear about the major metabolites of hydrolytic degradation of chlorotoluron in the water. 3-(3-chloro-p-tolyl)-1-methylurea and chlorotoluron benzoic acid are the main two metabolites which found in water and its sediments (WHO, 2003). Cleavage ring and demethylation, oxidation of chlorotoluron is observed in water (Tixier et al., 2000).

Chlorotoluron is not readily biodegradable and does not accumulate in the water and/or sediments in the water (WHO, 2003). When concentration of active substance in the water sediment has been identified above 10 % at $\geq$ 14 days, its metabolites have not been identified at the same case.

Due to chlorotoluron's mobility along soil profile it might found in surface and ground water. While, detected chlorotoluron concentration in drinking - water was 0.1µg l^{-1}, the guideline value for drinking - water for chlorotoluron is 0.03 mg l^{-1}. There is only very limited exposure to chlorotoluron to food (WHO, 2003).

Chlorotoluron is known as contaminant of groundwater.

Tab. 2: Physical and chemical characteristics of pesticide found in ground water (Damia and Marie, 1997) compared to chlorotoluron characteristics.

characteristic	Leaching criteria	Chlorotoluron
Water solubility	>30 ppm	74 ppm
Henry's Law constant	$< 10^{3}$ Pa (m^{-3}) (mol^{-1})	Very low
Hydrolysis half-life	>25 weeks	>52 weeks
Photolysis half-life	>1 week	>52 weeks
Soil adsorption: K_d	<1-5 (usually <1-2)	
Partition coefficient: K_{oc}	<300-500	log K_{oc} 2.5 ± 0.1
Aerobic soil metabolism half-life	>2-3 weeks	Longer
Field dissipation half-life	>2-3 weeks	Longer
Depth of leaching in field dissipation	>75-90 cm	

Once chemical contaminates groundwater, not easy maintenances are needed to purify it, while it is not stable but moving to all directions exceptional to leaching of non permeable aquitards and aquicludes. Chlorotoluron never detected in rain water Eastern France from year 2002-2003 (Anne et al., 2007).

3.5. Chlorotoluron fate and living organism

So far, the discussion has focused on chlorotoluron degradation in soil, water and air. However, living organisms may also play a significant role in chlorotoluron distribution and fate. Chlorotoluron degradation can take place by living organism is recorded by several authors (e.i. Tomohide Uno et al., 2010). In recent years, the biological analysis reveals that chlorotoluron readily accumulates in vegetables and plants. Thus, sorption, mobility, transformation of chlorotoluron, have received particular attention (Song et al., 2008).

As recorded in the study of Song et al., (2007), the chlorotoluron not only inhibits weed growth bus also accumulates in the plant root than the leaf elongation when it enhanced with increasing concentration of mixed amount of chlorotoluron to the soil. The study showed that high concentration of chlorotoluron was depressed some enzymes such as CAT and APT, in spite of POD, one of the oxidative indicator of higher plants, activity was increased in the root and leaves of wheat. Which means that chlorotoluron might get became growth inhibiting toxic chemical in high concentration (Song et al., 2007).

Metabolism of chlorotoluron and other phenylurea herbicides by fish CYP1 (cytochrome P450) family showed demethylation of chlorotoluron (Tomohide et al., 2010).

The transformation product (3-(3-chloro-p-tolyl)-methylurea) of chlorotoluron at concentration of 697 mg kg^{-1} showed harmful (see Appendix III) effect to earthworms, while the parent herbicide toxicity is not classified (Miller, P. and Westra P., Herbicide behavior in soil. Crop series, No 0.562 <http://cospl.coalliance.org/fez/eserv/co:5589/ucsu2062205621998 internet.pdf>).

3.6. Chlorotoluron transport

Chlorotoluron transport and mobility in the soil profile strongly depends on composition of soil structure which is reflected in soil structure and the configuration of the soil porous system, the aggregate stability, and soil hydraulic properties. When type and horizons of soils has different structure, its porosity and water flow which carries water soluble organic and inorganic substances are transported unevenly (Kodesova et al., 2008). The pores, part of the rock or soil space, which is not filled by the solid phase but with water and air and their formation, shape and size can be studied in different scales: micromorphometric and macromorphologic. It also influences soil saturated hydraulic conductivity (K_s) so called coefficient of filtration. Impact of macro porous system of the soil on K_s was explored by researchers in 1977 (Bouma et al., 1977). K_s values for soil which have different management practices were studied by (Pagliai et al., 1983), (Pagliai et al., 2003). Not only K_s but also effects of gravitational and big capillary pores are detectable in micromorphometric images were described by Kodesova et al., (2008) Through these pores water can move both horizontally and vertically together with pesticide and other water soluble substances, which can be transported from one place to another and from top layer of the soil down to groundwater. This can be described by preferential flow in the macropores of the soil.

Transport process in uneven distributed in entire soil (Kocarek et al., 2005) and (Kodesova et al., 2009). Due to organic matter and microorganisms where adsorption and degradation process is higher, the transport in top layer of the soil is slower than the deeper layers. The transport process is studied by (Kodesova et al., 2005), (Kodesova et al., 2008) and in the both top and deep layer (Kodesova et al., 2009). Chlorotoluron is highly mobile in saturated condition in deeper layer of well developed prismatic structured soil zone (B1 horizon of Haplic luvisol) (Kodesova et al., 2008).

4. MATERIALS AND METHODS

4.1. Soil preparation

The soil used for experiment was defined as Haplic Chernozem and air-dried to create equal condition for at the beginning of the bucket experiment in the greenhouse. Each buckets filled with 1120 g of soil whose stones, plant residues and other extrinsic objects which might influence the adsorption and degradation of chlorotoluron are removed during the drying step. Special textile which has high permeability is embedded at the bottom of the buckets to prevent soil to run out from the bucket. During filling the buckets soil has been mechanically pressed to avoid enable of big pores where plenty amount of water could be stored during the irrigation process.

4.2. Seeds and sowing

Three plants seeds – common wheat (*Triticum aestivum*), poppy (*Popaver rhoeas.L*) and weed (*Geranium pusillum*) - are selected for the experiment considering as basis of their viability and growth rate. For common wheat - 16, poppy – 5 x 3 (holes x seeds) and *Geranium pusillum* – 15 seeds were sown in each buckets. The number of seeds to be sown is estimated 3 times higher than the field condition (reality) according to estimation of bucket surface.

4.3. Application of chlorotoluron

The commercially available "Syncuran 80DP" which has 80 percent of active ingredient with concentration of 45.2 mg l^{-1} which has rate of 2.5 kg ha^{-1} pesticide applied pre-emergently and followed by washing with 50 ml of deionised water to rinse inoculating equipment wall where could remain pesticide residue in adsorbed form. Inoculating equipment was handmade which is designed to spread pesticide solution evenly on the soil surface and with constant flow rate. After application of pesticide buckets positioned randomly to decrease the handling error of plant grows and randomizing the green house conditions such as sunlight, shadow, evapotranspiration ect. Buckets are irrigated only from the bottom, which was the most suitable irrigation procedure for the experiment, to avoid leaching of cholotoluron out of the soil. Sufficient amount of water was added during all along the experiment. Amount of the water which added to each buckets is shown in Appendix IV. In the later part of the experiment, when plants completely welt, irrigation has stopped.

4.4. Sampling and sample preparation

The entire soil samples which were in buckets are taken 1, 7, 15, 24, 35, 49, 74, 97 and 200 days after the start of the experiment with 3 replicates from each plant species. Entire soil samples whose plant roots separated are lyofilized, ground, sieved through 2 mm sieve and homogenized prior to the extraction by methanol.

Note: The last 2 sampling (97 and 200 day after application of chlorotoluron) have taken without plant root separation from the soil.

10 g of soil sample was weighted with accuracy of ± 0.002 g and 10 ml of grade methanol was added to glass tube with screw cap, then followed by intensive shaking for 24 hours in the room temperature to extract the chlorotoluron molecule. After shaking suspension has transferred to plastic centrifugal tube and centrifuged at the 13000 rotation per minute during 30 minutes in the cooling centrifuge at 5^{o}C. Then the extract passed by microfilter which would not allow the solid particles to enable to enter the HPLC system. Chlorotoluron in methanol extract has analysed by means of HPLC system with UV detector against the analytical chlortoluron standards.

4.4.1. Materials and instruments

- Buckets
- Permeable geotextile
- Freezer
- Lyofiliser
- Grinder
- Sieve, with 2 mm pores
- Homogeniser
- Glass tube with screw cap
- Analytical balance with accuracy of 0.0001 g
- Shaker
- Centrifuge
- Centrifugal tube with screw, plastic
- Small glass ware for HPLC (vials)
- Filter
- Syringe
- HPLC instrument (explained in 4.5.)
- Statictical software – StatGraphic

4.4.2. Reagents and solvents

All chemicals and solvents used were of analytical reagent grade. All reagents were from Lach-Ner (Neratovice, Czech Republic). The pesticide standards used in this study were from Sigma Aldrich.

- Deionised water
- Methanol
- Formic acid
- Acetonitrile
- Chlorotoluron standard, Sigma Aldrich

4.5. Measurement and HPLC Condition

The determination of chlorotoluron in water and methanol extracts were performed using HPLC instrument. This instrument consists of the following parts: P680 HPLC Pump, ASI-100 Automated Sample Injector. The columns (differ for water and methanol extracts) were placed in the Thermostatted Column Compartment TCC-100 set to a constant temperature of 25 °C. Detection of chlorotoluron was performed by PDA-100 Photodiode Array Detector. The signal from the detector was stored and processed using the chromatographic software Chromeleon version 6.70 (Dionex). The separation of methanol extracts took place in Kinetex 2.6, C18 column 100 A, 50 x 4.6 mm (Phenomenex). The guard column (Security Guard Cartridge AQ C18 4 x 2.00 mm) were used for to prolong the lifetime of the column. The mobile phase for chlorotoluron in methanol extracts were: A) 20% acetonitrile and 80 % deionised water and B) 80% acetonitrile and 20 % deionised water. Both A and B mobile phase were buffered by formic acid in the concentration of 1ml l^{-1}. The constant flow was set to 1 ml min^{-1}. The multigradient steps for determination of Chlorotoluron in methanol extract is given in Tab. 3. The wavelength of chlorotoluron detection was 252 nm and the retention time was 2.95 min.

Tab. 3: Retention time and wavelengths used for pesticide determination in methanol extracts

Herbicide name	Retention time (min)	B ratio (%)
Chlorotoluron	0	20
	0	20
	2	20
	4.5	80
	6	100
	7.5	100
	8	20
	12	20
	13	end

5. RESULTS

5.1. Half-life of chlorotoluron

Measured chlorotoluron concentration in each soil samples are shown in Appendix V. The average concentration of chlorotoluron for each variant is shown in Fig. 5.

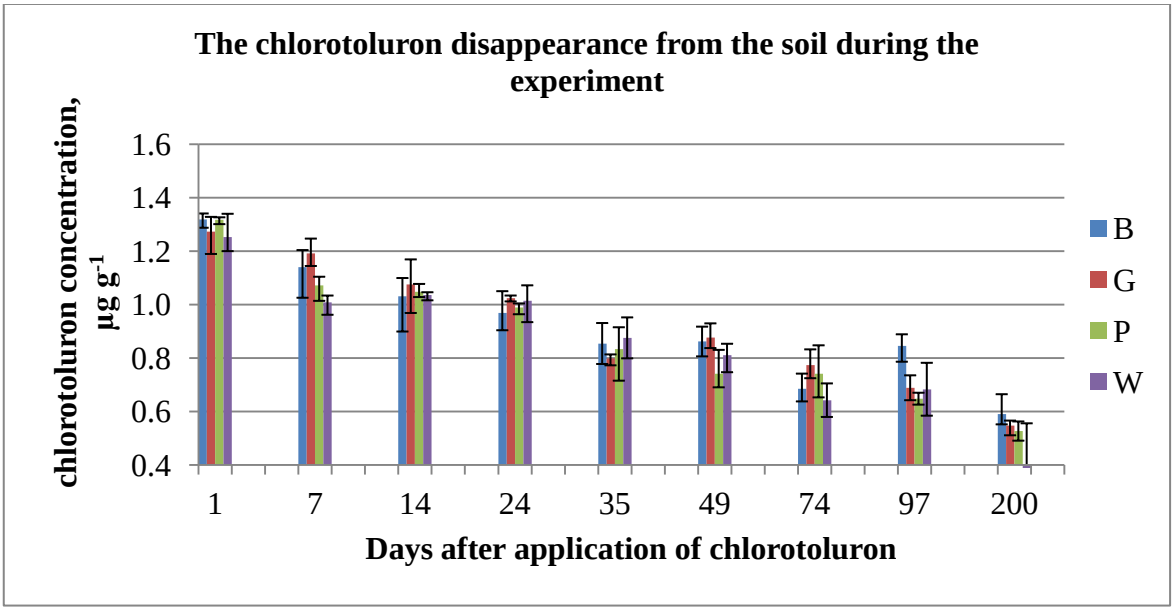


Fig. 5: The concentration of chlorotoluron during the experiment. Where: B - blank P – poppy (*Papaver rhoesas.* L), G – weed (*Geranium pusillium*) and W -wheat (*Triticum aestivum*).

The chlorotoluron disappearance from the soil by time was observed. The degradation intensity was rapid at the beginning of the experiment, at the later part it slowed down. Later on factors might have influenced for extended disappearance of the chlorotoluron from the soil will discussed in the discussion part.

The cholotoluron half-life was determined by using first-order rate equation (1) and half-life (2) (see 3.1.) for soil where each variant of growth was grown and blank soil sample. Figures for each case are shown below. The rate constants were different for each variant and blank.

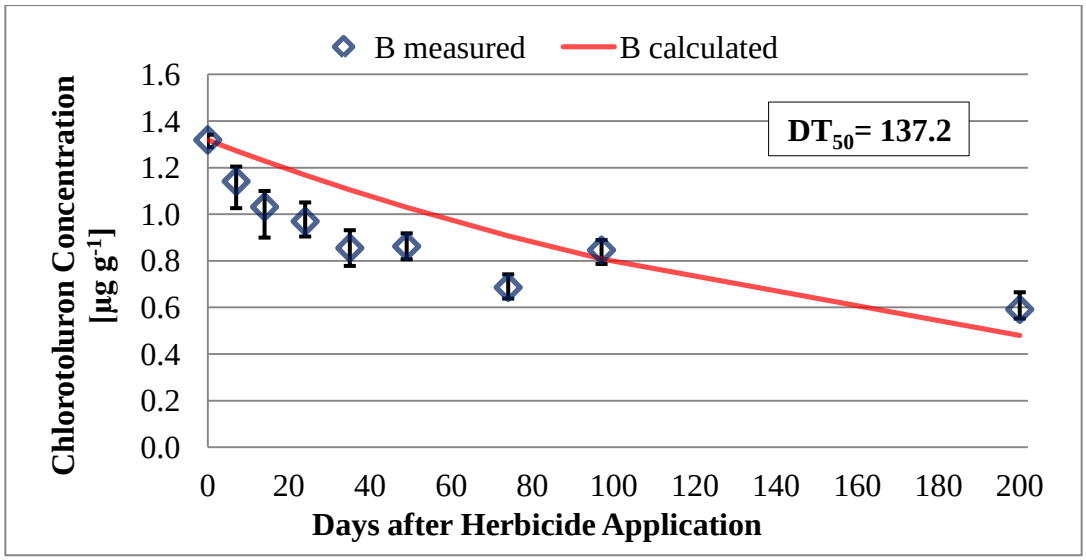


Fig. 6: Measured and calculated half-life of chlorotoluron in the blank samples. The half-life of chlorotoluron was 137.2 days.

Slight increase of chlorotoluron concentration 97 days after application of chlorotoluron was observed in blank s samples cannot be explained in any way, but some experimental error could have occurred while handling the soil samples.

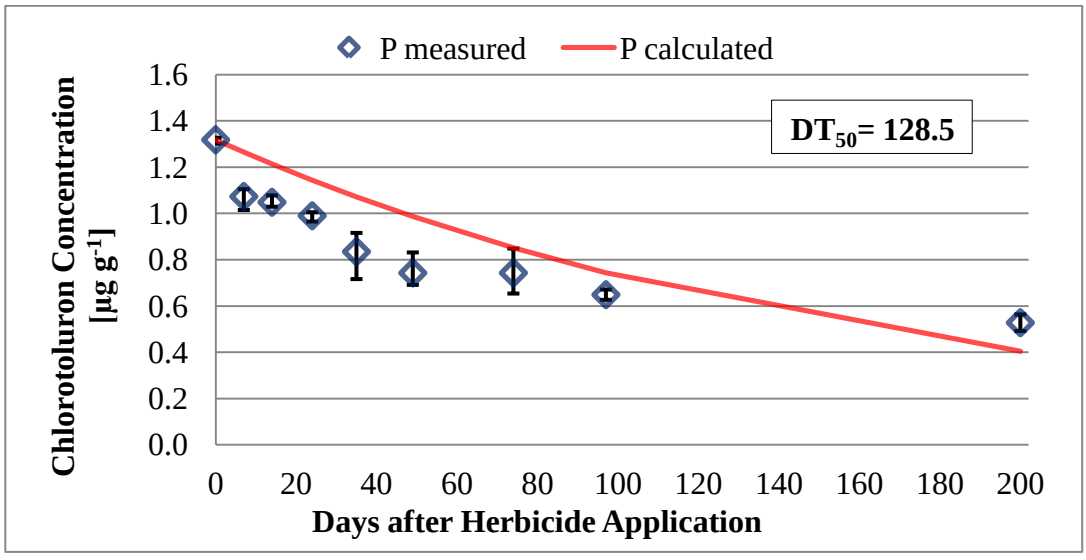


Fig. 7: Measured and calculated half-life of chlorotoluron in the soil where *Papaver rhoesas*. L (poppy) was grown. The Half-life of chlorotoluron was 128.5 days.

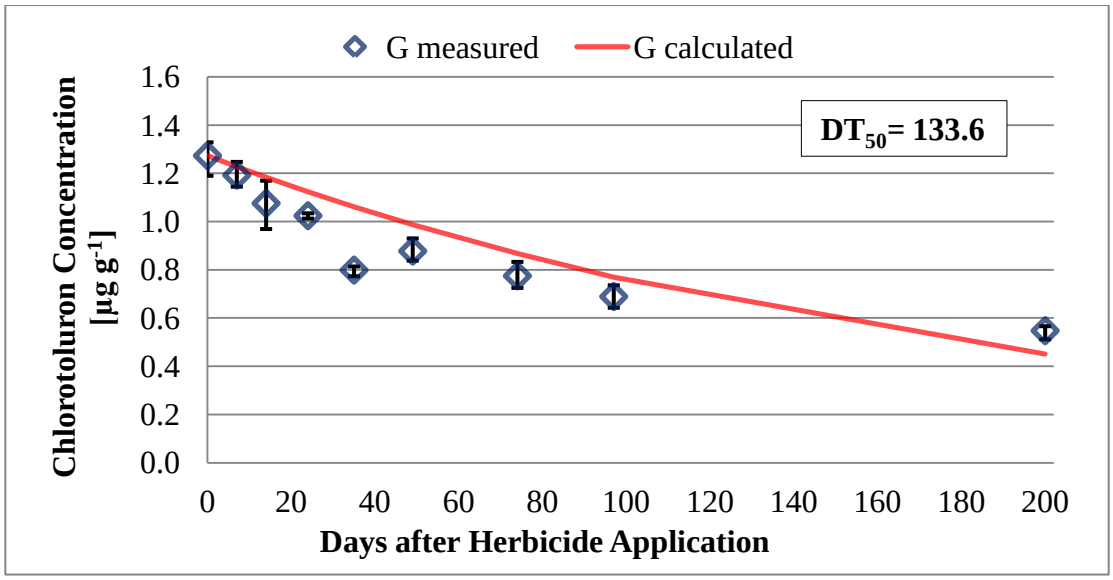


Fig. 8: Measured and calculated half-life of chlorotoluron in the soil where *Geranium pusillium* (weed) was grown. The half-life of chlorotoluron was 133.6 days.

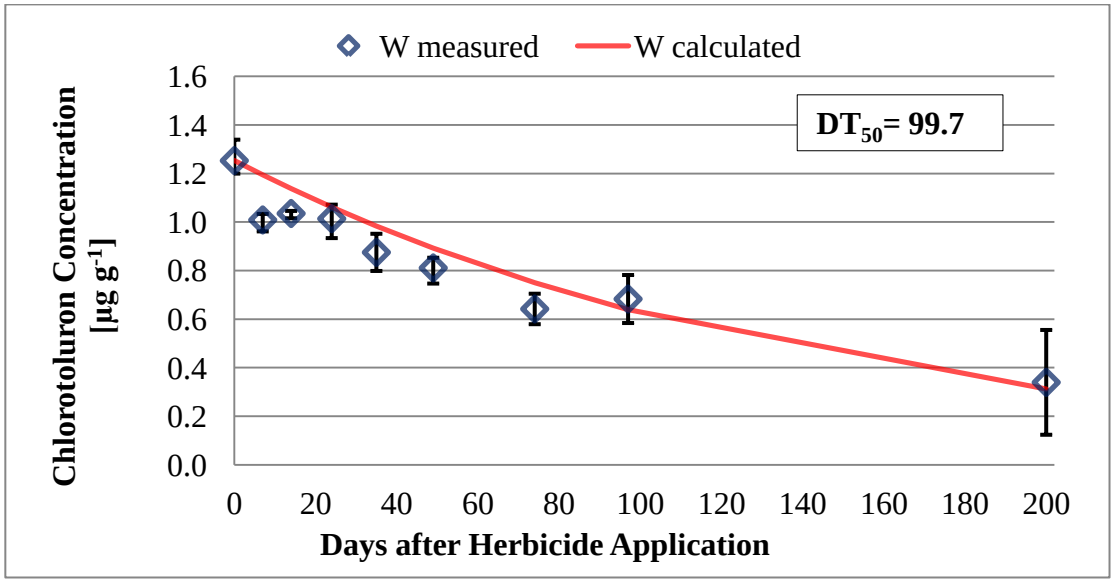


Fig. 9: Measured and calculated half-life of chlorotoluron in the soil where *Triticum aestivum* (wheat) was grown. The half-life of chlorotoluron was 99.7 days.

During the experiment the *Triticum aestivum* (wheat) developed the densest room system. The slight increase in chlorotoluron concentration at 97 days after application might be explained by accumulation of chlorotoluron in the wheat root. The sampling from 1 to 7 the root system was completely separated from the soil which we could not effort it in last two sampling.

The half-life of the chlorotoluron 200 days after application ranged between 99.7 days to 137.2 days. The shortest half-life for this study was found for soil where the *Triticum aestivum* (wheat) was grown, then followed by *Papaver rhoesas.* L (poppy) - 128.5 days, *Geranium pusillium* (weed) - 133.6 days and the longest half-life was found in blank samples with 137.2 days. The chlorotoluron degradation rate decreased as follow: Blank > Poppy > Weed > Wheat

5.2. Statistical outputs

5.2.1. Growth influence on chlorotoluron disappearance

To check the significant difference on effect of growth variants and blank sample on chlorotoluron disappearance from the soil, using StatGraphics, Centurion Data Analysis and Statistical Software was run.

The significant difference from each means was determined by using multiple comparison procedure and estimated difference between each pair of means are shown in the Tab. 4 and 5, respectively. The method which has been used to discriminate among the means was Fisher's least significant difference (LSD) procedure. With this method, there was 5 % risk of calling each pair of means significantly different when the actual difference equals 0.

Tab. 4: Output of applied multiple range tests. Where: B – blank, P – poppy (*Papaver rhoesas.* L), G – weed (*Geranium pusillium*) and W -wheat (*Triticum aestivum*)

The growth type	*Count*	*Mean*	*Homogeneous Groups*
P	29	0.869203	X
W	26	0.890515	X
B	25	0.934372	X
G	29	0.940121	X

The homogenous group is identified by a column of X's. There were no statistically significant differences between any pair of means at the 95 % confidence level. Within each column, the levels containing X's form a group of means within which there are no statistically significant differences.

Tab. 5: Output of estimated difference between each pair of means. Where: B – blank, P – poppy (*Papaver rhoesas.* L), G – weed (*Geranium pusillium*) and W - wheat (*Triticum aestivum*)

Contrast	*Sig.*	*Difference*	*+/- Limits*
B - G		-0.00574869	0.128004
B - P		0.0651686	0.128004
B - W		0.0438566	0.131378
G - P		0.0709172	0.123172
G - W		0.0496053	0.126675
P - W		-0.0213119	0.126675

There are no statistically significant differences between any pair of means at the 95 % confidence level. However there is difference between DT_{50} values ranging from 99.7 days for wheat to 137.2 days for blank, no statistically differences were found between the plant variants and the blank sample with 95 % of confidence interval.

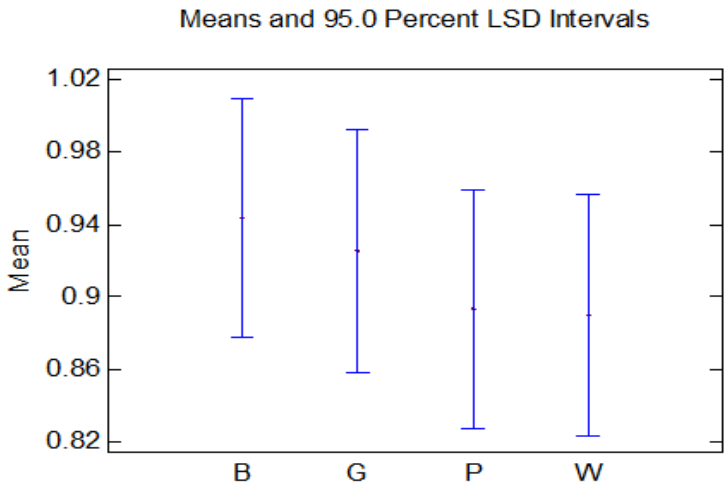


Fig. 10: The range of chlorotoluron concentrations during the experiment. Where: B – blank, P – poppy (*Papaver rhoesas.* L), G – weed (*Geranium pusillium*) and W - wheat (*Triticum aestivum*)

5.2.2. The disappearance of chlorotoluron in each variant at each sampling day

The disappearance of chlorotoluron in each variant at each sampling day was tested by Multi-Sampling Comparison test.

Tab. 6: Effect of growth between variants and blank sample on disappearance of chlorotoluton from the soil at each sampling day.

Day after application	P-Value	Day after application	P-Value
1	0.4176	49	0.138
7	**0.0389**	74	0.2817
14	0.8972	97	0.1128
24	0.6024	200	0.2332
35	0.7938		

Only the 7 days after application of chlorotoluron gave significant difference between growths studied. Its P-value was <0.05, equal to 0.0389, which represents significant difference at 95 % confidence. See Tab. 7 and 8 for detail information about outputs.

Tab. 7: Output of Multi-Sampling Comparison test, 7 day after application of chlorotoluron. Where: B – blank, P – poppy (*Papaver rhoesas.* L), G – weed (*Geranium pusillium*) and W - wheat (*Triticum aestivum*)

	n	*Mean*	*Homogeneous Groups*
W	3	1.00917	X
P	3	1.07247	XX
B	3	1.14083	X
G	3	1.19223	X

The homogenous group is identified by a column of X's. There were statistically significant differences between means at the 95 % confidence level. Within each column, the levels containing X's form a group of means within which there were statistically significant differences.

Tab. 8: Output of estimated difference between each pair of means, 7 day after application of chlorotoluron. Where: B – blank, P – poppy (*Papaver rhoesas.* L), G – weed (*Geranium pusillium*) and W - wheat (*Triticum aestivum*)

Contrast	*Sig.*	*Difference*	*+/- Limits*
B - G		-0.0514	0.12239
B- P		0.0683667	0.12239
B -W	*	**0.131667**	0.12239
G - P		0.119767	0.12239
G -W	*	**0.183067**	0.12239
P -W		0.0633	0.12239

* denotes a statistically significant difference

The concentration of chlorotoluron in each studied variant samples, 7 days after application showed significant difference. Significant differences were found between pairs blank – wheat and weed – wheat.

5.2.3. Chlorotoluron concentration vs. Growths

The correlation between concentration of chlorotoluron and growths was statistically analyzed by simple regression and fitted to the linear model (Y = a + b*X, where Y - chlorotoluron concentration (dependant variable), a - intercept, b - slope, X - growths (independent variable)). The output is shown in the Fig. 11.

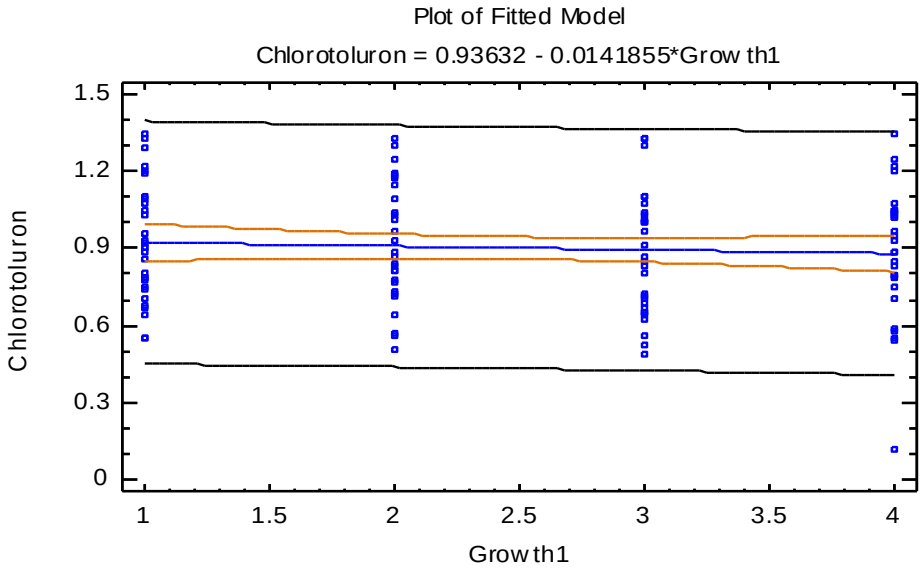


Fig. 11: Plot of fitted model of Chlorotoluron concentration vs. Growths. Correlation Coefficient = -0.07, R-squared = 0.4 %, P-Value = 0.4658

There were relatively weak negative correlation (correlation coefficient = -0.07) between chlorotoluron concentration and growths. The 0.4 %, (R-squared value) of the chlorotoluron variable fitted to the linear model. This means that disappearance of chlorotoluron from the soil was not effected by growths at 95 % confidence level.

5.2.4. Influence of irrigation on chlorotoluron disappearance from each variant

The significant difference of irrigation influence on chlorotoluron disappearance was analysed.

Tab. 9: Output of Multi-Sampling Comparison test on irrigation influence on each variants
Where: B – blank, P – poppy (*Papaver rhoesas.* L), G – weed (*Geranium pusillium*) and W - wheat (*Triticum aestivum*)

Method: 95.0 percent LSD

	Count	*Mean*	*Homogeneous Groups*
B	7	29.5429	X
G	7	33.9	X
P	7	34.6429	X
W	7	47.4857	X

The homogenous group is identified by a column of X's. There were no statistically significant differences between any pair of means at the 95 % confidence level. Within each column, the levels containing X's form a group of means within which there are no statistically significant differences.

Tab. 10: Output of estimated difference between each pair of means, irrigation influence on each variant, Where: B – blank, P – poppy (*Papaver rhoesas.* L), G – weed (*Geranium pusillium*) and W -wheat (*Triticum aestivum*)

Contrast	*Sig.*	*Difference*	*+/- Limits*
W - P		12.8429	20.7445
W - G		13.5857	20.7445
W - B		17.9429	20.7445
P - G		0.742857	20.7445
P - B		5.1	20.7445
G - B		4.35714	20.7445

* denotes a statistically significant difference.

The irrigation effect on in each studied variant samples did not show significant difference.

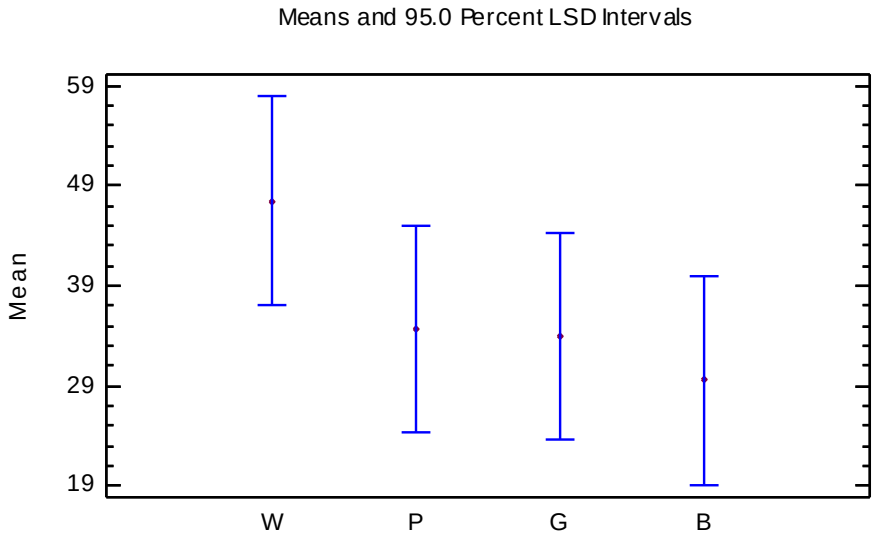


Fig. 12: The range of irrigated water during the experiment. Where: B – blank, P – poppy (*Papaver rhoesas.* L), G – weed (*Geranium pusillium*) and W - wheat (*Triticum aestivum*)

5.2.5. Chlorotoluron concentration vs. Irrigation

Decrease in chlorotoluron concentration depending on irrigation was tested and was fitted to linear regression model (Y = a + b*X, where Y - chlorotoluron concentration (dependant variable), a - intercept, b - slope, X - irrigation (independent variable)).

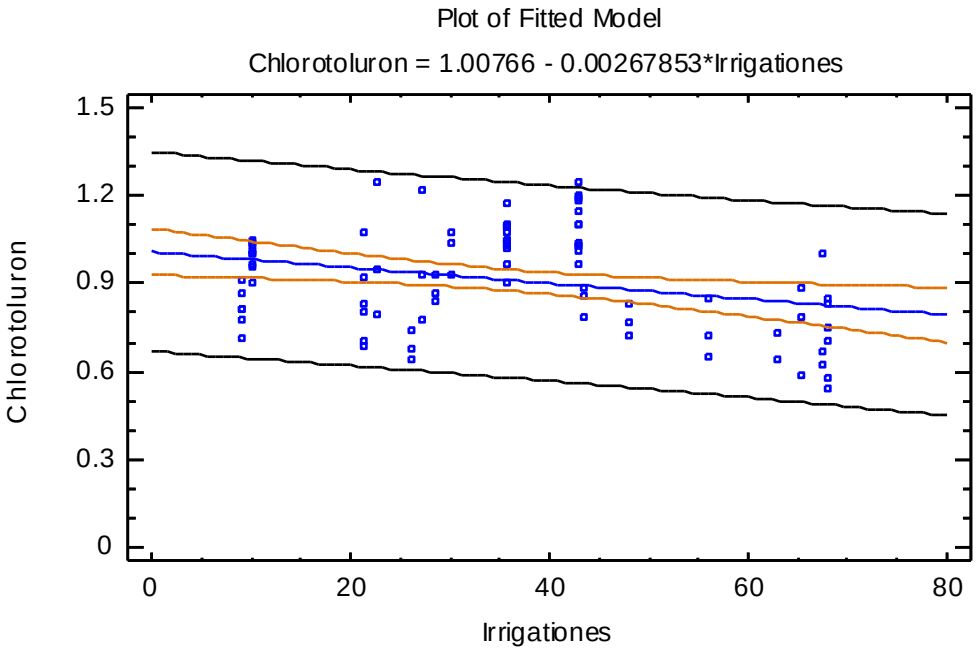


Fig. 13: Plot of fitted model of Chlorotoluron concentration vs. Irrigation. Correlation Coefficient = -0.29, R-squared = 8.4 %, P-Value = 0.0079

There were relatively weak negative correlation (correlation coefficient = -0.29) between chlorotoluron concentration and irrigation. The 8.4 % (R-squared value) of the chlorotoluron variable fitted to the linear model. This means that disappearance of chlorotoluron from the soil was effected by irrigation at 95 % confidence level.

5.2.6. Chlorotoluron concentration vs. Time (day)

The change in concentration of chlorotoluron by time was statistically analyzed by simple regression and fitted to the linear model (Y = a + b*X, where Y - chlorotoluron concentration (dependant variable), a - intercept, b - slope, X - time (day) (independent variable)). The output is shown in the Fig. 14.

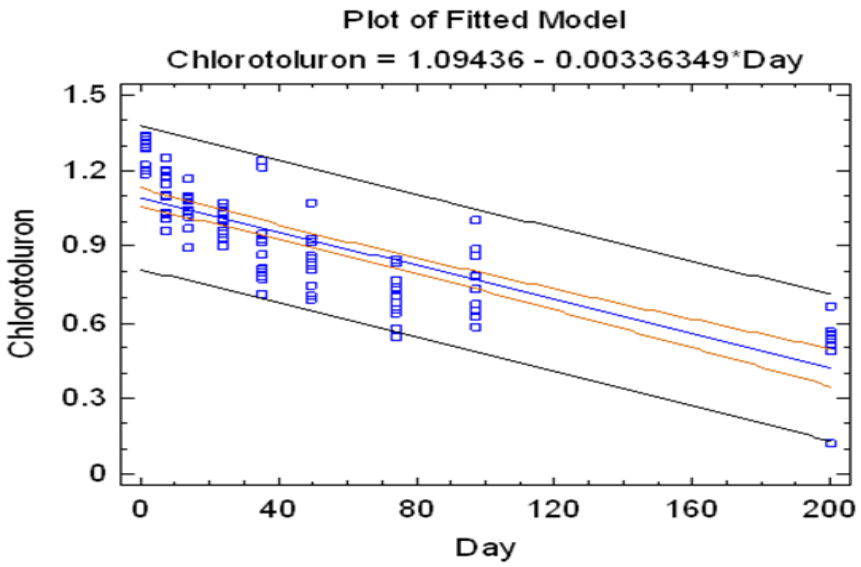


Fig. 14: Plot of fitted model of Chlorotoluron concentration vs. Time (day). Correlation Coefficient = -0.81, R-squared = 65.7 %, P-Value=0.0000

There were moderately strong negative correlation (correlation coefficient = -0.81) between chlorotoluron concentration and time (day). The 65.7 % (R-squared value) of the chlorotoluron variable fitted to the linear model. This shows that disappearance of chlorotoluron from the soil was effected by the time (day) at the 95 % confidence level.

The effect of growths and irrigation on disappearance from the soil was tested by multifactor ANOVA also showed the similar results (see Tab. 11) as results shown above. The chlorotoluron disappearance was effected by irrigation (p-value = 0.0000) but not by growths (p-value = 0.2411) at 95 % confidence level.

Tab. 11: Analysis of Variance for Chlorotoluron vs. Growths and Irrigation

Source	*Sum of Sq*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Growth	0.0453677	3	0.0151226	1.44	**0.2411**
B:Irrigation	1.73249	17	0.101911	9.67	**0.0000**
RESIDUAL	0.653248	62	0.0105363		
TOTAL	2.43419	82			

6. DISCUSSION

6.1. Half –life of chlorotoluron

The disappearance of the chlorotoluron from the soil, half-life and influence of the plant species to chlorotoluron degradation is studied. Quantitative understanding of the kinetic of degradation for different organic compound in soils would be very valuable as this would help in the prediction of persistence. The ideal soil-applied herbicide would persist long enough to control weed growth, but not so long to remain in soil after harvesting the crop. Persistence of the chlorotoluron in the soil profile is influenced by factors such as soil type, mobility and transport (Kocarek et al., 2005), weather condition (Hance, 1980).

Many other studies, which targeting to determine chlorotoluron half-life obtained varying research outcomes depending on the study conditions, climate factors and objective of experiment. Smith and Griggs, (1978) determined half-life time of chlorotoluron 28 to 42 days while Blume et al. found 42 to 126 days. Also soil types gave different approach which 93 days for silty sand soil and 40 days for silty loam soil is reported in Rudel et al's study. The DT_{50} value was varying wide due to the experimental conditions (ex: temperature, presence of oxygen), where the experiment is performed whether in laboratory or in field. Chlorotoluron had half-life of 13 to 92 days in aerobic laboratory condition at 20^0C, in contrast to anaerobic condition which has DT_{50}value of 591 days (Directive 91/414/EEC). Kocarek et al., (2010) found chlorotoluron has half-life of 35 days. Half-life of chlorotoluron in 0 - 10 cm of the top soil, which was not treated with chlorotoluron in the past, was 64 days, but in soil from plot which was annually treated with chlorotluron for 13 years gave half-life of only 11 days (Tixier et al., 2000).

Different half-lives of chlorotoluron were found depending on soil type, biomass and horizon. For cambisol, Borstel DT_{50} values ranged between 27-493 days, for cambisol, Ebbinghof value ranged from 22-117 days (Kordel et al, 1995). Degradation of chlorotoluron was biomass and depth-dependent in the subsoil.

In this study the half-life calculated 200 days after application of chlorotoluron was higher than compared to those previous half-life studies in laboratory condition. But the

half-life determined 74 days after application of chlorotoluron (from same research), which presented in the Student Scientific Conference, 2011, at Wageningen University, The Netherlands, was comparable to those studies published. The result 74 days after chlorotoluron application showed 69.2 days, 66.3 days, 75.7 days and 62.0 days for blank, poppy, weed and wheat respectively (see Appendix VI).

However, microorganism are able to degrade wide variety of chemicals, from simple to complex structured organic compounds, their activity depends on temperature and pH. Generally, the temperature accepted as optimal for the activity of microorganisms indigenous to soil range between 20-30^{0}C. Tariq et al., (2006) reported that increasing temperature (15 - 35^{0}C) was shortened half-life of pesticide but was not significant difference found between sterile and non-sterile soils. Thus, the temperature is not a direct factor for defining the pesticide degradation, but influences microorganisms in the soil involved degradation process. The degradation rates of some organochlorine pesticides (e.i. aldrin, dieldrin, endrine) in constant temperature (at 30^{0}C) were much higher and half-lives were 3-5 times shorter than for the same soil under variable temperature of outdoor condition (Ghadiri et al., 1995). Ghadiri et al., (1995) study result proved the microorganism importance to degradation process. The extended half-life of chlorotoluron in our study can be explained by high temperature in the greenhouse during the experiment. High temperature in the greenhouse, effected negatively to microbial activity. While, the microorganism cannot carry out their metabolism and synthesis of enzymes (protein deactivation), activity of degradation was decreased.

It was interesting that Tariq et al., (2006) reported high half – life in sterile (autoclaved) soil was not as significant as non-sterile soils in the laboratory condition. They explained the reason may be the low organic carbon content, insufficient amount of nutrients and ultimate source of microbial activity. Conclusion from their study, the temperature, along with humidity, plays major role in degradability of pesticide in sandy loam soil of the cotton-growing area of Punjab, Pakistan.

Just as temperature level stimulate different soil microbes, so does soil moisture. Difference in soil moisture seems to have a more intensive effect on the degradation of chemical than differences in soil temperature (Anastasiah et al., 2011). Kocarek et al., (2010) reported that the preferential flow through macropores in the soil give fluctuation of herbicide concentration along the soil profile. Also the majority of chlorotoluron

concentration found at the top 2-4 cm of the soil was observed in the same study. The evident influence of both temperature and moisture enhanced pesticide degradation in the soil is observed (Tariq et al., 2006). In our study, it is possible to say that high temperature in the soil increased the evaporation from the soil and evapotranspiration rate, and limited the interaction between the microbial communities with chlorotoluron molecule. Correct irrigation regime decreases persistence (increase degradation rate) of chemicals and accumulation even in the deep layer soil (Manika et al., 2010). But the sensitivity of pesticides degradation rate to soil moisture varies between pesticides to pesticide (Ghadiri et al., 1995). Degradation rate of some pesticides (organochlorines) were not effected by higher water content in the soil (Ghadiri et al., 1995).

For most chemicals just a certain amount of these coumpounds are bioavailable and only this portion can be degraded by microorganisms (Katamaya et al., 2010). The microbial breakdown is not only dominated by the activity of the microorganisms but also the mass transfer of pesticide to microbes and the prevalent water regime in soils (Han and New, 1994). The distribution of pesticide and pesticide degrading microbes are uneven in the soil, and pesticides must mostly diffuse to the more or less immobile microbes to be metabolized by them. Hampered mass transfer of chemical to the degrading microbes could therefore be a limiting factor in biodegradation (Hance, 1980). Thus, soil moisture is one of the important parameters regulating bioavailability and degradation (Anastasiah et al., 2011). At the end of our experiment availability of chlorotoluron was degreased to concentration approximately 0.34-0.59 μg g^{-1}. The decrease of chlorotoluron degradation in the later part of the experiment can be explained by the fact bioavailability of the chlorotoluron in the soil sample was limited.

The next reason for extended half-life of chlorotoluron might be change of natural soil condition to perform experiment in the greenhouse. Studies of the fate of herbicide in soil must be carried out using soil in condition that approaches those in the field as closely as possible. Soil collected for use in the studies should be handled as living tissue rather than as geological specimen. Practices such as air-drying, prolonged storage, and freezing and thawing will drastically alter the biochemical capability by inactivating extracellular enzyme and by modifying the composition and density of population of microorganisms indigenous to soils (Hance, 1980).

This study results might give a great opportunity to understand and planning of experiment duration and importance of climatic conditions and soil properties, which influence the half-life of chlorotoluron. Finding of different half-lives of chlorotoluron, 74 and 200 days after application, for each variant of soil samples were prove of the fact that persistence of chemical is not chemical-specific, but it highly influenced by soil type and climatic conditions at given time and site where and when the study was performed.

6.2. Statistics

The both results from 74 and 200 days after application of chlorotoluron did not show significant statistical difference at 95 % of confidence interval, the chlorotoluron disappearance from the soil was not effected by the types of plant. However, the half-life for wheat grown soil sample was shorter than the other variants and blank in both cases. It might explained by high evapotranspiration rate (required more water) which followed by more frequent irrigation gave opportunity to increase water in the soil. Also the dense rooting system might have increased the uptake of chlorotoluron from the soil. Opposite results have obtained for blank samples whose half-life was the highest (137.2 days). Increase in degradation rate might have influenced by experimental change in frequency of irrigation followed by the lack of water in the soil.

The chlorortoluron concentration vs. time, growth and irrigation was statistically analysed by simple regression. It was clear that chlorotoluron concentration will have moderately strong correlation with time. Fitted values for chlorotoluron concentration to linear model were 65.7% for time. Same correlation was found between chlorotoluron concentration and irrigation. Fitted value was 8.4 %. But no correlation for growths was observed. The regression analysis was made at 95 % confidence level.

Null hypothesis "H_0 - The plant variants have influence on degradation of chlorotoluron", is rejected at 95 % of confidence level. There was no significant influence between plant variants to chlorotoluron degradation rate. But for each sampling case, chlorotoluron concentration for each variant was affected only after 7 days of application. The difference was found between samples with blank – wheat (*Triticum aestivum*) and weed (*Geranium pusillium*) – wheat (*Triticum aestivum*).

Next null hypothesis "H_0 - The irrigated water have influence on degradation rate (half-life of herbicide)" was not rejected.

7. CONCLUSION AND RECOMMENDATION

Influence of plant growth on chlorotoluron disappearance from the soil cannot be easily deduced from the simplified greenhouse experiment. While, chlorotoluron is applied in different climatic conditions all over the world, in different climate and soil type, its half-life depends on many other factors. Conclusion of this diploma thesis can be summarized by following:

- The half-life of chlorotoluron was 99.7 days for *Triticum aestivum* (wheat), 128.5 days for *Papaver rhoesas*. L (poppy), 133.6 days for *Geranium pusillium* (weed) and 137.2 days for blank samples. The half –lives determined from our study at given condition was longer compared to common reported half-lives.
- The plant growths had no effect on chlorotoluron disappearance from the soil in the greenhouse condition.
- Irrigation had effect on disappearance of chlorotoluron from the soil for each variant of samples. But irrigated water volume had no correlation with chlorotoluron disappearance.

8. LIST OF LITERATURE

AISLABIE, J and Lloyd-Jones, G. 1995, A review of bacterial degradation of pesticide. Australian Journal of Soil research, vol.33, p. 925-942.

ALAN, N. 1993, Partition coefficients (n- octanol- water) for pesticides. Journal of Chromatography A, Vol. 642, no. 1-2, p. 3-14.

ANASTASIAH, N., Ulrike, D. 2011, Hagen, S., Zachary, G., Hamadi, B., Reiner, S. Effect of fluctuating soil humidity on in situ bioavailability and degradation of atrazine. Chemosphere, vol. 84, no. 4, p. 369-375.

ANNA, S., Stephane, M., Phillippe, M., Maurice, M. 2007, Pesticide analysed in rainwater in Alsace region (Eastern France): Comparison between urban and rural sites. Atmospheric Environment, vol. 41, no. 34, p. 7241-7252.

BAATE, E and Anderson, T.H. 2003, Comparison of soil/bacterial ratios in a pH gradient using physiological and PLFA-based techniques. Soil Biology and Biochemistry, vol. 35, p. 955-963.

BENNY, C. 2004, Yitzhak, B., Tamara, P. Sorption-desorption behaviour of triazine and phenylurea herbicides in Kishon river sediments. Water Research, vol. 38, no. 20, p. 4383-4394.

BEULKE, S., Brown, C.D. 2004, Fryer, C.J, Van Beanum, W. Influence of kinetic sorption and diffusion on pesticide movement through aggregate soils, Chemosphere, vol. 57, p. 481-490.

BOUMA, J., Jongerius, A., Boersma, O., Jager, A., Schoonderbeek, D. 1977, The function of different types of macropores during saturated flow through four swelling soil horizons. Soil Science. Soc, AM.J., vol. 41, p. 945-950.

BRICENO, G., Palma, G., Duran, N. 2007, Influence of organic amendment on the biodegradation and movement of pesticides. Critical Review of Environmental Science and Technololgy. vol. 37, no. 3, p. 233–271.

BURROWS, H.D., Canle, L.M., Santaballa, J.A., Steenken, S. 2002, Reaction pathways and mechanism of photodegradation of pesticides, Journals of Photochemistry and Photobiology B:Biology, vol. 67, no. 2, p. 71-108.

CARABAIS-MARTINEZ, R., Rodriguez-Gonzalo, E., Herrero-Hernàndez, E., Sànchez-San Romàn, F.J., Prado Flores, M.G. 2002, Determination of herbicides and metabolites by solid-phase extraction and liquid chromatography. Evaluation of pollution due to herbicides in surface and groundwaters. J. Chromatography, A, vol. 950, p. 157–166.

CASTILLO, M.A., Felis, N., Aragon, P., Cuesta, G., Sabater, C. 2006, Biodegradation of the herbicide diuron by streptomyces isolated from soil. International Biodeterioration and Biodegradation, vol. 58, no. 3-4, p. 196-202.

CHAPMAN, R.A., Harris, C.R. 1990, Enhanced degradation of insecticide of pesticide in the environment. American Chemical Society, Washington, DC, p. 82-96.

DAMIA, B. And Marie-Claire, H. 1997, *Trace determination of pesticideand their degradation product in water*. Amsterdam, The Netherlands: Elsivier Science.B.V, p. 1 – 78, ISBN 0-444-81842-1.

FAO Soils Bulletin 80, The importance of soil organic matter, Key to drought-resistant soil and sustained food production. p. 1-34

FIELD, J.A., Reed, R.L, Sawyer, T.E., Griffith, S.M., Wigington, P.J. Jr. 2003, Diuron occurrence and distribution in soil and surface and ground water associated with grass weed production. Environmental Qualilty, vol. 32, p. 171–179.

Finalised in the Standing Committee on the Food Chain and Animal Health at its meeting on 15 February 2005 in view of the inclusion of chlorotoluron in Annex I of Directive 91/414/EEC. Available at: <http://ec.europa.eu/food/plant/protection/evaluation/existactive/list_chlortoluron.pdf>

FRANZLUEBBERS, A.J. 2002, Water infiltration and soil structure related to organic matter and its stratification with depth. Soil Tillage Res., vol. 66, p. 97–205.

Garcia-Orenes.F., Guerrero.C., Mataix-Solera.J., Navarro-Pedreño.J., Gómez.I.,

MATAIX-BENEYTO, J. 2005, Factors controlling the aggregate stability and bulk density in two different degraded soils amended with biosolids. Soil Tillage Res., vol. 2, no. 1, p. 65–76.

GARY, D., 2007, Bending, M., Sonia, R.C. Microbial aspect of the interaction between soil depth and biodegradation of the herbicide isoproturon. Chemosphere, vol. 66, no. 4, p. 664-671.

GHADIRI, H., Rose, C.W., Connel, D.W. 1995, Degradation of organochlorine pesticide in soil under controlled environment and outdoor conditions. Journal of Environmental Managemnet, vol. 43, no. 2, p. 141-151.

GROEN, K.P. 1997: Pesticide leaching in polders. Fields and model studies on cracked clays and loamy sand. Van ZEE tot Land 62, Leystad, ISBN 90-369-1209-1, p. 296.

HAN, O.S and New, P.B. 1994, Effect of water availability on degradation of 2,4-dichlorophenoxyacetic acid (2,4-D) by soil microorganisms. Soil Biology and Biochemistry, vol. 26, p. 1689-1697.

HEMMAT, A., Aghilinategh, N., Rezainejad, Y., Sadeghi, M. 2010, Long-term impacts of municipal solid waste compost, sewage sludge and farmyard manure application on organic carbon, bulk density and consistency limits of a calcareous soil in central Iran. Soil Tillage Res., vol. 108, no. 1–2, p. 43–50.

HOUOUT, S., Top, E., Yassir, A., Soulas, G. 2000, Dependance of accelerated degradation of atrazine on soil pH in French and Canadian soils. Soil Biology and Biochemistry, vol. 32, p. 615-625.

JAVIER BENITEZ, F., Francisco, J.Real., Acero, L. Carolina, G. 2006, Photochemical oxidation processes for the elimination of phenylurea herbicides in waters. Journals of Hazardous Material, vol. 138, no. 2, p. 278-287.

JIANBO, L., Chuanfan, Q. 1995, Hydrophobic coefficient of s-atrazine and phenylurea herbicides. Chemosdphere, vol. 31, no. 8, p. 3951-3959.

KATAMAYA, A., Bhula, R., Burns, G.R., Carazo, E., Felsot, A., Hamilton, D., Harris, C., Kim, Y.-H., Kleter, G., Koedel, W., Linders, J., Peijnenburg, J.G.M.W., Sabljic, A., Stephenson, R.G., Packe, D.K., Rubin, B., Tanaka, K., Unsworth, J., Wauchope, R.D. 2010, Bioavailibilty of xenobiotics in the soil environment. Review of Environmental Contamination, vol. 203, p. 1-86.

KOCAREK, M., Kodesova, R., Kozak, J., Drabek, O., Vasek, O. 2005, Chlorotoluron behaviour in five different soil types. Plant, Soil an Environment, vol.51, no.7, p. 304-309.

KOCAREK, M., Kodesova, R., Kozak, J., Drabek, O. 2010, Field study of chlorotoluron transport and its prediction by the BPS mathematical model. Soil Water Resources, vol. 5, no.4, p. 153-160.

KODESOVA, R., Kocarek, M., Hajkova, T, Hybler, M., Drabek, O., Kodes, V., 2012, Chlorotoluron mobility in compost amended soil, Soil Tillage Research, vol. 118, no. 1, p. 188–96.

KODESOVA, R., Vignozzi,. N., Rohoskova, M., Hajkova, T., Kocarek, M., Pagliai, M., Kozak, J., Simunek, J. 2009, Impact of varying soil structures on soil transport processes in different diagnostic horizons of three soil types. Journal of Contaminant Hydrology, vol. 104, no. 1-4, p. 107-125.

KODESOVA, R., Kocarek, M., Kodes, V., Simunek, J, Kozak, J. 2008, Impact of soil micromorphological features in water flow and herbicide transport in the soils. Vadose Zone, 7 (2), 7 (2), vol. 7, no. 2, p. 798-809.

KODESOVA, R., Kozak, J., Simunek, J., Vacek, O. 2005, Single and dual- permeability model of chlorotoluron transport in soil profile. Plant Soil Environment, vol. 51, no. 7, p. 310-315.

MANIKA, G., Garg, N.K., Himanshu, J., Sharma, M.P. 2010, Persistence and mobility of 2,4-D in unsaturated soil zone under winter wheat crop in sub-tropical region of India. Agriculture, Ecosystem and Environment, vol. 146, no. 1, p. 60-72.

MANUAL, A.E., Eugenio, L.P., Elena, M.C., Jesus, S.G., Juan, C.M., Luis, G.R. 2008, The mobility and degradation of pesticide in soil and the pollution of groundwater resources. Agriculture, Ecosystem and Environment, vol. 123, no. 4, p. 247-260.

MEYER-WINDEL, S., Ketelsen, H., Widmoser, P. 1997, On the relation of herbicide adsorption and soil organic fraction. Z.Pflanz. Bodendunde, vol. 160, no. 4, p. 361-368.

MILLET, M., Palm, W.U., Zetzsch, C. 1998, Investigation of the photochemistry of urea herbicide (chlorotluron and isoproturon) and quantum yields using polychromatic irradiation. Environmental Toxicological Chemistry, vol. 17, p. 258-264.

NORA, B., Stig, R., Stefan, O., Birthe, B.K., Andres, H.J., Ole, S.J., Jens, A. 2009, Metabolites of the phenylurea herbicide chlorotoluron, diuron an linuron produced by the soil fungus *Mortierella* sp. Environmental pollution, vol. 157, no. 10, p. 2806 - 2812.

PAGLIAI, M., Marca, M.La., Lucamante, G. 1983, Micromorphic and micromorphological investigations of a clay loam soil in viticulture under zero and conventional tillage. Soil Science, vol. 34, p. 391-403.

PAGLIAI, M., Marsili, A., Servado, P., Vignozzi, N., Pellegrini, S. 2003, Changes in some physical properties of clay soil in central Italy following the passage of rubber tracked and wheeled tractors of medium power. Soil Tillage Res., vol. 79, p. 119-129.

PAGLIAI, M., Vignozzi, N., Pellegrini, S. 2004, Soil structure and the effect of management practices. Soil Tillage Res., vol. 79, no. 2, p. 131–143.

SMITH, AE., Griggs, GG. 1978, Weed Research, vol. 18, no. 1-7, p. not available.

SONG, N.H., Chen, L, Yang, Y. 2008, Effect of dissolved organic matter on mobility and activation of chlorotoluron in soil and wheat. Geodema, vol. 146, p. 344- 352.

SONG, N.H., Yin, C.L., Chen, G.F., Yang, H. 2007, Biological response of wheat (*Triticum Aestivum*) plants to the herbicide chlorotoluron in soils. Chemosphere, vol. 68, no. 9, p. 1779-1787.

SONG, N.H., Zhang, S., Hong, M., Yana, H. 2010, Impact of dissolved organic matter on bioavailability of chlorotoluron to wheat. Environmental pollution, vol. 158, no. 3, p. 906-912.

SPARK, K.M., Swift, R.S. 2002, Effect of soil composition and dissolved organic matter on pesticide sorption. Science of The Total Environment, vol. 298, no. 1-3, p. 147-161.

TARIQ, M.I., Afzal, S., Hussain, I. 2006, Degradation and persistence of cotton pesticides in sandy loam soils from Punjab, Pakistan. Environmental Research, vol. 100, no. 2, p.184-196.

TEJADA, M., Gonzalez, J.L. 2006, The relationships between erodibility and erosion in a soil treated with two organic amendments. Soil Tillage Res., vol. 91, no. 1–2, p. 186–198.

TEJADA, M., Hernandez, M.T., Garcia, C. 2009, Soil restoration using composted plant residues: effects on soil properties. Soil Tillage Res., vol. 102, no. 1, p. 109–117.

TIXIER, C., Sancelme, M., Ait-Aisse, S., Widehem, P., Bonnemoy, F., Cuer, A., Truffaut, N., Veschambre, H. 2002, Biotransformation of phenylurea herbicides by soil bacterial strain, *Arthrobacter* sp. N2: structure, ecotoxicity and fate of diuron metabolites with soil fungi. Chemosphere, vol. 46, no. 4, p. 519-526.

TIXIER, C., Meunier, L., Bonnemoy, F., Boule, P. 2000, Phototransportation of three herbicide: chlorbufam, isoproturon, and chlorotoluron. Influence of iiradaiation on toxicity. International Journal of Photoenergy, vol. 2.

TOMOHIDE, U., Satoru, K., Tatsushi, G., Hiromasa, I., Masahiko, N., Kengo, K., Hiroshi, Y., Yoshio, K., Takao, I. 2011, Metabolism of the herbicides chlorotoluron, diuron, linuron, simazine, and atrazine by CYP1A9 and CYP1C1 from Japanese eel (*Anguilla japonica*). Pesticide Biochemistry and Physiology, vol. 101, no. 2, p. 93-102.

VANHALA, P., Karhu, K., Tuomi, M., Bjorklof, K., Fritze, H., Liski, J. 2008, Temperature sensitivity of soil organic matter decomposition in southern and northern areas of the boreal forest zone. Soil Biology and Biochemistry, vol. 40, no. 7, p. 1758-1764.

VISCHETTI, C., Monaci, E., Cardinali, A., Casucci, C., Perucci, P. 2008, The effect of initial concentration, co-application and repeated application on pesticide degradation in a biobed mixture. Chemosphere, vol. 72, no. 11, p. 1739-1743.

WHO (2003) Chlorotoluron in drinking-water. Background document for preparation of WHO Guidelines for drinking-water quality. Geneva, World Health Organization (WHO/SDE/WSH/03.04/33).

YANG, H, Wu, X, Zhou, L.X, Yang, Z.M. 2005, Effect of dissolved organic matter on chlorotoluron sorption and desorption. Pedosphere, vol. 15, no. 4, p. 432- 439.

ZEBARTH, B.J., Neilsen, G.H., Hogue, E., Neilsen, D. 1999, Influence of organic waste amendments on selected soil physical and chemical properties. Canadian Journal of Soil Science, vol. 79, p. 501–504.

Ziv, A., Cilia, L.Fuentes. 2007, Accelerated biodegradation of pesticide: An overview of the phenomenon, its and possible solutions; and a discussion on the tropical dimension Crop protection, vol. 26, no. 12, p. 1733-1746.

9. APPENDIX

Common name (ISO)	Chlorotoluron
Chemical name (IUPAC)	3-(3-chloro-p-tolyl)-1,1-dimethylurea
Chemical name (CA)	*N*-(3-chloro-4-methylphenyl)-N,N-
CIPAC No	217
CAS No	15545-48-9
EEC No	2395922
FAO SPECIFICATION	217/TC/S (1990) (Content shall be declared not less than 975 $g.kg^{-1}$ (+/- 20
Minimum purity	975 $g.kg^{-1}$
Molecular formula	$C_{10}H_{13}ClN_2O$
Molecular mass	212.7 $g.mol^{-1}$
Melting point	148.05°C
Boiling point	Not applicable
Appearance	Colourless crystals, odourless
Relative density	$\rho = 1.34 \times 10^3$ $kg.m^{-3}$ at 22°C
Vapour pressure	5×10^{-6} Pa at 25.0°C
Henry's law constant	Not determined due to very low vapour
Solubility in water	74 mg/l at 25^0C and 20^0C (PPDB)
Solubility in organic solvents	Ethanol: 48 $g.l^{-1}$ (25°C) Acetone:54 $g.l^{-1}$ (25°C) Toluene :3.0 $g.l^{-1}$ (25°C) n-Octanol:24 $g.l^{-1}$ (25°C) n-Hexane:60 $g.l^{-1}$ (25°C) Ethyl Acetate:21 $g.l^{-1}$ (25°C) Dichloromethane:51 $g.l^{-1}$ (25°C)
Partition coefficient (log P_{ow})	2.5 ± 0.1 (25^0C pH=7)
Hydrolytic stability (DT_{50})	pH 5: Stable (> 1 yr). pH 7: Stable (> 1 yr). PH 9: Stable (> 1 yr).

Appendix 1: Identity, physical and chemical properties of chlorotoluron, (WHO, 2003)

Dissociation constant	No dissociation constant is available in an accessible pH range
Quantum yield of direct photo-transformation in water at λ >290 nm	Not applicable
Flammability	Not flammable
Explosive properties	No self-ignition.: Chlorotoluron is not thermally sensitive, shock sensitive nor friction sensitive
UV/VIS absorption (max.)	The molar absorptivity was determined to be 19516 $M^{-1}cm^{-1}$ at 241-242 nm
Photostability in water (DT_{50})	Chlorotoluron photolysis followed first order kinetics yielding a rate constant of 6.87 x $10^{-5}s^{-1}$, half-life 2.8 hours (Hg lamp λ > 190 nm)

Continuous of Appendix 1: Identity, physical and chemical properties of chlorotoluron, (WHO, 2003)

Descriptive terminology	Acidity	Base	Water solubility	Volatility	Soil retention*	Persistence
	pH	pH	mg l^{-1}	mm Hg x 10^{-6}		Days
Very low or very slow	<2	>8	<10	<1	$<10^2$	<10
Low or slow	3-4	6-8	$10\text{-}10^2$	1-10	$10^2\text{-}10^3$	10-30
Moderate	4-6	4-6	$10^2\text{-}10^3$	$10\text{-}10^2$	$10^3\text{-}10^4$	30-90
High or long	6-8	3-4	$10^3\text{-}10^4$	$10^2\text{-}10^3$	$10^4\text{-}10^5$	90-180
Very high or very long	>8	<2	$>10^4$	$>10^3$	$>10^5$	>180

* Higher numbers mean greater soil retention.

Appendix II: Adjectives description to the base or acid strength, water solubility, volatility, soil retention and soil persistence. (Miller, P. and Westra P., Herbicide behavior in soil. Crop series, No 0.562 Available at: <http://cospl.coalliance.org/fez/eserv/co:5589/ ucsu2062205621998internet.pdf>)

Classification	Level of chemical in soil ($mg\ kg^{-1}$)
Very toxic	≤50
Toxic	50 <x≤250
Harmful	250<x≤1000
Not classified	>1000

Appendix III: Hazard classification for acute toxicity for soil dwelling organisms. Miller, P. and Westra, P., Herbicide behavior in soil. Crop series, No 0.562, Available at: <http://cospl.coalliance.org/fez/eserv/co:5589/ucsu2062205621998internet.pdf>

Date	Day	Volume of irrigated water (ml)				Sampling
		Wheat	Poppy	Weed	Blank	
29.3 2011	0	200	200	200	200	
30/03/2011	1					1st
01/04/2011	3	100	100	100	100	
05/04/2011	7					2nd
07/04/2011	9	250	250	250	250	
12/04/2011	14	150				3rd
18/04/2011	20	150	100	100	100	
22/04/2011	24				200	4th
28/04/2011	30	150				
30/04/2011	32	100	100	100	100	
03/05/2011	35	200				5th
06/05/2011	38	150	150	150	150	
08/05/2011	40	200				
10/05/2011	42	150	150	150	150	
12/05/2011	44	250				
15/05/2011	47			100		
17/05/2011	49	250	150	150	150	6th
19/05/2011	51	150	250			
21/05/2011	53	250		150		
24/05/2011	56	250	250	250	250	
27/05/2011	59	250				
29/05/2011	61		250	250		
31/05/2011	63	250			250	
04/06/2011	67		250	250		
06/06/2011	69	150	100			
08/06/2011	71	150	150	150		
11/06/2011	74	250	150	150	250	7th

Appendix IV: Irrigation water volume for each variant samples

15/06/2011	78	250	150	150		
18/06/2011	81	250	250	250	250	
21/06/2011	84	250	250	250	250	
23/06/2011	86	250	250			
26/06/2011	89			250	250	
28/06/2011	91	250	250	150		
30/06/2011	93		250	250		
04/07/2011	97					8th
06/07/2011	99	250	250	250	250	
15/10/2011	200					9th
		5500	**4250**	**4050**	**3150**	

Continuous of Appendix IV: Irrigation water volume for each variant samples.

	Sample Name	Ret.Time	Area	Height	Amount
		min	mAU*min	mAU	µg/g
1	W1	2.9583	1.7445	12.9959	**1.3399**
2	W2	2.9500	1.5625	11.5830	**1.2001**
3	W3	2.9500	1.5891	11.8760	**1.2205**
4	W4	2.9500	1.3429	10.0529	**1.0315**
5	W5	3.0083	1.2525	9.1494	**0.9620**
6	W6	3.0083	1.3463	9.9098	**1.0340**
7	W7	2.9500	1.3624	10.2113	**1.0464**
8	W8	3.0083	1.3600	9.9493	**1.0446**
9	W9	2.9167	1.3229	9.9160	**1.0161**
10	W10	3.0083	1.3495	9.8650	**1.0365**
11	W11	3.0083	1.3959	10.3013	**1.0721**
12	W12	3.0083	1.2166	8.8845	**0.9344**
13	W13	2.9500	1.0403	7.7942	**0.7990**
14	W14	3.0083	1.6211	11.9608	**1.2451**
15	W15	2.9167	1.2398	9.4374	**0.9522**
16	W16	2.9500	1.0859	8.1084	**0.8341**
17	W17	2.9500	1.1112	8.2990	**0.8535**
18	W18	2.9500	0.9727	7.2527	**0.7471**
19	W19	2.9583	0.9182	6.7895	**0.7052**
20	W20	2.9500	0.7105	5.2769	**0.5457**
21	W21	3.0083	0.7547	5.4735	**0.5797**
22	W22	3.0083	1.1537	8.4757	**0.8861**
23	W23	2.9500	0.7610	5.6926	**0.5845**
24	W24	2.9583	1.0185	7.5232	**0.7823**
25	W25	2.8917	0.7004	5.1499	**0.5559**
26	W26	2.9000	0.1565	1.0627	**0.1242**
27	W27	2.8833	0.9844	7.2990	**0.7813**

Appendix V: Measured chlorotoluron concentration in soil sample where wheat (*Triticum aestivum*-W) was grown

	Sample Name	Ret.Time	Area	Height	Amount
		min	mAU*min	mAU	µg/g
1	P1	2.9500	1.7251	12.7875	**1.3250**
2	P2	3.0000	1.7273	12.7546	**1.3267**
3	P3	3.0000	1.6940	12.4983	**1.3011**
4	P4	2.9167	1.4378	10.7977	**1.1043**
5	P5	3.0083	1.3203	9.7100	**1.0140**
6	P6	2.9583	1.4310	10.6636	**1.0991**
7	P7	2.9500	1.4027	10.4473	**1.0774**
8	P8	2.9500	1.3388	9.8560	**1.0283**
9	P9	2.9583	1.3506	10.0345	**1.0373**
10	P10	2.9583	1.3005	9.6780	**0.9989**
11	P11	2.9500	1.3078	9.7839	**1.0045**
12	P12	3.0083	1.2551	9.1888	**0.9640**
13	P13	2.9167	0.9315	6.9898	**0.7154**
14	P14	3.0083	1.1342	8.3628	**0.8711**
15	P15	2.9500	1.1917	8.8838	**0.9153**
16	P16	3.0083	0.9175	6.7478	**0.7047**
17	P17	2.9500	0.8990	6.7226	**0.6905**
18	P18	2.9500	1.0815	7.9751	**0.8307**
19	P19	2.9500	0.8501	6.3648	**0.6529**
20	P20	3.0000	0.9452	6.9136	**0.7260**
21	P21	2.9500	1.1037	8.1756	**0.8477**
22	P22	2.9167	0.8147	6.1121	**0.6258**
23	P23	2.9583	0.8726	6.5111	**0.6702**
24	P24	2.9500	1.3083	9.7757	**1.0049**
25	P25	2.8833	0.6186	4.6170	**0.4910**
26	P26	2.8833	0.6636	4.8725	**0.5267**
27	P27	2.8833	0.7092	5.2339	**0.5629**

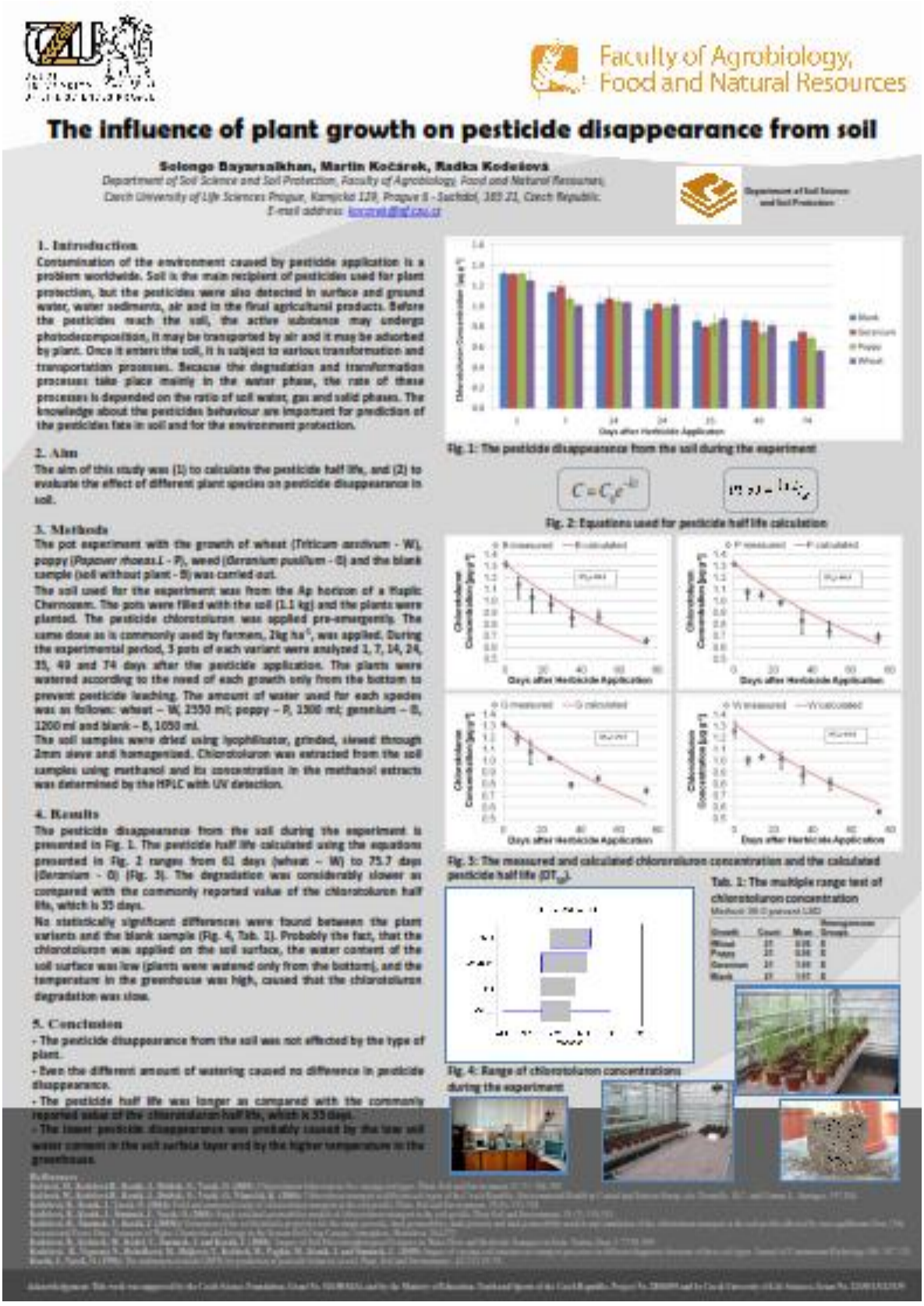
Continuous of Appendix V: Measured chlorotoluron concentration in soil sample where poppy (*Popaver rhoeas.L*-P) was grown

	Sample Name	Ret.Time	Area	Height	Amount
		min	mAU*min	mAU	µg/g
1	G1	2.9167	1.7294	12.9266	**1.3282**
2	G2	3.0083	1.5488	11.3938	**1.1896**
3	G3	2.9500	1.6936	12.6616	**1.3008**
4	G4	2.9500	1.6237	11.9840	**1.2471**
5	G5	2.9583	1.4902	11.0081	**1.1445**
6	G6	2.9500	1.5430	11.4338	**1.1851**
7	G7	2.9250	1.2612	9.4765	**0.9687**
8	G8	2.9500	1.5222	11.3334	**1.1692**
9	G9	3.0083	1.4164	10.4184	**1.0878**
10	G10	3.0000	1.3354	9.8536	**1.0256**
11	G11	2.9500	1.3463	9.9100	**1.0340**
12	G12	2.9583	1.3176	9.6996	**1.0120**
13	G13	2.9500	1.0594	7.8543	**0.8136**
14	G14	2.9167	1.0062	7.4998	**0.7728**
15	G15	3.0083	1.0534	7.7186	**0.8091**
16	G16	2.9583	1.0904	7.9540	**0.8375**
17	G17	2.9500	1.1257	8.3652	**0.8646**
18	G18	2.9583	1.2107	8.8933	**0.9299**
19	G19	2.9583	1.0839	8.0424	**0.8325**
20	G20	2.9500	0.9435	7.0690	**0.7246**
21	G21	2.9583	0.9978	7.3625	**0.7664**
22	G22	2.9500	0.9521	7.1053	**0.7313**
23	G23	2.9583	0.8363	6.2178	**0.6423**
24	G24	2.9375	1.1060	8.9045	**0.8495**
25	G25	2.8833	0.7132	5.2576	**0.5660**
26	G25	2.8833	0.7110	5.3318	**0.5643**
27	G27	2.8833	0.6440	4.7227	**0.5111**

Continuous of Appendix V: Measured chlorotoluron concentration in soil sample where weed (*Geranium pusillum* - G) was grown

	Sample Name	Ret.Time	Area	Height	Amount
		min	mAU*min	mAU	µg/g
1	B1	2.9500	1.6764	12.5043	**1.2876**
2	B2	2.9500	1.7460	12.9714	**1.3410**
3	B3	2.9583	1.7279	12.9252	**1.3271**
4	B4	2.9500	1.5530	11.6747	**1.1928**
5	B5	2.9500	1.3354	9.9798	**1.0256**
6	B6	3.0083	1.5677	11.4359	**1.2041**
7	B7	3.0083	1.4316	10.6724	**1.0995**
8	B8	3.0000	1.4252	10.5662	**1.0946**
9	B9	2.9500	1.1709	8.7977	**0.8993**
10	B10	2.9500	1.1771	8.7924	**0.9041**
11	B11	3.0083	1.3675	10.1065	**1.0503**
12	B12	2.9500	1.2409	9.1263	**0.9531**
13	B13	2.9500	1.2124	9.0241	**0.9312**
14	B14	3.0083	1.0127	7.4411	**0.7778**
15	B15	2.9583	1.5851	11.7134	**1.2174**
16	B16	2.9500	1.1947	8.9053	**0.9176**
17	B17	2.9500	1.3990	10.3104	**1.0745**
18	B18	2.9500	1.0499	7.7724	**0.8063**
19	B19	2.9583	0.8304	6.1914	**0.6378**
20	B20	2.9583	0.9658	7.1112	**0.7418**
21	B21	2.9583	0.8817	6.5229	**0.6772**
22	B22	2.9167	1.1228	8.3976	**0.8624**
23	B23	2.9500	1.0238	7.5272	**0.7864**
24	B24	2.9583	1.1574	8.5391	**0.8889**
25	B25	2.8833	0.7011	5.2776	**0.5565**
26	B26	2.8833	0.6955	5.0899	**0.5520**
27	B27	2.8917	0.8377	6.1079	**0.6648**

Continuous of Appendix V: Measured chlorotoluron concentration in blank samples



Appendix VI: Poster presentation presented at the Student Scientific Conference at Wageningen University, The Netherlands.