INVESTIGATION OF LEACHING PROCESSES OF HERBICIDES IN SOIL COLUMN SIMULATING BY HYDRUS-1D MODEL

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Abstract

A leaching experiment focusing on atrazine and metolachlor was conducted using a soil column with prescribed hydrological conditions. The transport parameters determined the concentrations in the soil depth. Atrazine and metolachlor were applied at the surface of the soil column at the average concentrations of 2.23±0.04 and 2.15±0.02 mg kg^{-1}, for atrazine and metolachlor, respectively. At the end of the experiment, the concentrations of atrazine and metolachlor in the soil surface were 0.0068±0.0015 and 0.0013±0.0012 mg kg^{-1}, respectively. The concentrations in atrazine and metolachlor in the water percolating at the bottom of the column were 0.14±0.03 mg kg^{-1} and 0.21±0.01 mg kg^{-1}, respectively. The computed dissipation rate constants ranged from 0.014 to 0.036 day^{-1} for atrazine and from 0.010 to 0.028 day^{-1} for metolachlor. The corresponding half-lives were 23.5 days and 24.7 days for atrazine and metolachlor, respectively. There were no significant differences in leaching nor in the dissipation behaviors between atrazine and metolachlor (Student's t-test, α = 0.05). The HYDRUS-1D simulated herbicide concentrations had fair accuracy, and the RMSE values between the simulated results and observed data were about 0.25 mg.L^{-1} for atrazine and 0.29 mg.L^{-1} for metolachlor. At the middle of the soil column, the soil water concentrations increased to maximum values of 2.24 and 2.98 mg.L^{-1} at 28 and 24 min and decreased to 0.31 and 0.36 mg.L^{-1} at the final time for atrazine and metolachlor,

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respectively. This result may be due to the very similar physicochemical properties of atrazine and metolachlor.

Keywords: Leaching processes, soil column, HYDRUS-1D, agricultural soil, herbicides

Introduction

Herbicides are applied to protect agriculture products. Inappropriate usage and management of herbicides will contaminate and damage the environmental resources. The prediction and assessment of the herbicides' fate and transport in soil is required to minimize adverse impacts in the environment. Many models have been developed to simulate and predict the fate and transport of agricultural chemicals. The most famous models are based on convection-dispersion equations and consider such mechanisms as convection, dispersion, sorption, and degradation. The transport of solutes, including herbicides, has been widely studied under field and laboratory conditions in both disturbed and undisturbed soil columns. The sorption and degradation of herbicides are included in studies (Van Genuchten and Wagenet, 1989; Singh *et al.*, 1994; Van Genuchten *et al.*, 2012). The simulation model of the leaching processes for predicting the herbicides' fate and transport in this study is HYDRUS-1D (Šimůnek *et al.*, 1999) which was developed in support of the HYDRUS model (Raymundo *et al.*, 2011). Simulation dependent parameters are the effects of temperature, biochemical degradation rate, and the soil adsorption coefficient (Ebato *et al.*, 2001). Both the water content and herbicides' concentration in successive soil layers were investigated by considering the herbicides' characteristics, soil properties, and climatic condition.

In this study, types of commonly found agricultural soils in Japan were used to evaluate the accuracy of the model regarding the prediction of soil water content. To improve the accuracy of the model, the degradation rates of atrazine and metolachlor were investigated in a laboratory experiment. Precise control of hydrological conditions in the leaching experiment enables the simplification of the environmental scenarios, while simulating the herbicides' behavior as in actual agricultural fields (Gevao *et al.*, 2000). Soil column leaching experiments are an important tool that can be used to study the migration of organic matter in soils. The fate and transport of herbicides in the environment is a complex phenomenon that depends on many factors such as temperature and water management. The simulation models have been developed for analyzing herbicides' fate and transport to assess the potential environmental risk. The HYDRUS-1D model considers temperature, water content, degradation rate, and soil dependency for hydrolysis, and microbial degradation.

The main objectives of this study were to investigate the solute transport of atrazine and metolachlor in a soil column using the HYDRUS-1D model.

Materials and Methods

Soil Column Leaching Experiments

The leaching experiments of both herbicides were conducted using soil columns with prescribed hydrological conditions under room temperature. The soil samples were prepared and packed into 35 cm long soil columns. The breakthrough curves of the atrazine and metolachlor were measured in 2 intact columns of agricultural soil. The soil for the columns was taken from the depth of surface soils (0-5 cm) located at Sakaecho (35°68'N, 63°9'E) by Tokyo University of Agricultural and Technology, Fuchu, Tokyo, Japan. The soils were filled into the 35 cm soil columns. The main components of the soil were sand and clay particles with the volume percent of 29.5% and 23.4%, respectively. The moisture content of the soil was 11.50%, and the organic matter was 6.95%, with a high specific surface area, and contained short-range ordered minerals. The particle size distribution, total organic carbon, pH, and cation exchange capacity results of the soil used in these experiments were recorded (Jaikaew *et al.*, 2015). After collecting the soil from the experimental farm, it was sieved with 2 mm diameter wire before visible roots and organic residues were removed and the soil air-dried. Then, the soil was transferred into stainless steel cylinders (length, 35 cm; diameter, 5 cm) in a total of 7 layers as shown in Figure 1. Duplicate leaching columns were packed with untreated, air-dried, and sieved soil (<2 mm) up to a height of approximately 35 cm. To obtain uniform packing, the soil was added to the columns in small portions with a spoon and pressed under simultaneous gentle column vibration until the top of the soil columns did not sink any further. To control the reproducibility of the packing procedure, the total weight of the soil was packed in the columns in a total of 7 layers.

After packing, in order to induce moisture saturation in the soil, 1 and 1/2 liters of the leaching solutions ($CaCl_2$: 0.01 mol L^{-1}), introduced from bottom to top in order to displace the air in the soil pores with water, was used to leach the soil columns at a flow rate of 2.5 $L \cdot d^{-1}$. After 24 h, the soil was saturated and soil was taken from the surface of the soil column and mixed with 10 g of atrazine std. (2.23 mg/kg) and metolachlor std. (2.12 mg/kg) and the application rates were 771.3 and 732.5 g.a/ha, respectively. Thereafter the soil columns were allowed to equilibrate and the excess water was drained off by gravity. The methods for the columns' saturation followed the guideline OECD 312 (Organization for Economic Cooperation and Development, 2004).

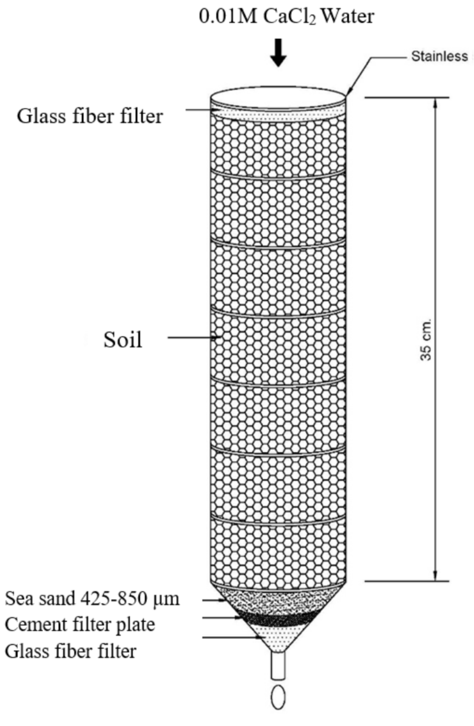

Figure 1. Schematic diagram of the soil column (high = 35 cm, diameter = 5 cm)

Materials of Herbicides

Atrazine and metolachlor (purity 99.5%, analytical standards and certified reference materials) were purchased from Wako Pure Chemical Industries (Osaka, Japan). HPLC-grade acetonitrile, acetone, ethyl acetate, diethylene glycol, sodium chloride, and calcium chloride (all GR grade) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Water was obtained with a Milli-Q purifier system (Merck Millipore, Billerica, MA, USA).

Water and Soil Sampling

Water samples were collected during the experiment using leaching collectors located at the lower end of the columns. The total amount of water in the column experiment measured 500 ml, and sub-samples were obtained for herbicide analysis every 30 min during the 3 h artificial leaching column experiment. After leaching, the soil was cut layer by layer of the 7 layers, and an amount of soil (10 g of soil for each layer) was tested for herbicides in the.

Herbicides Extractions

Water Sample

Water samples were extracted using the method in Jaikaew *et al.* (2015). Briefly, water samples were filtered through a glass fiber filter and then the pH was adjusted to 2.5 by phosphoric acid before extraction. Water samples of 500 ml were extracted twice with 50 ml of methylene chloride. The combined extracts were dried over anhydrous $NaSO_2$ and the eluate was evaporated to dryness under a gentle stream of nitrogen (40-45°C); the dry residue was re- suspended into 2 mL of acetonitrile, filtered through a 0. 22-µm polytetrafluorethylene (PTFE) filter, and then was analyzed in an HPLC-diode array detection (DAD) system as is described below.

Soil Sample

For atrazine and metolachlor extraction, 5 g of the soil sample were transferred to conical flasks, and mixed with 5 mL of water and 5 mL of CH_3CN(1% CH_3COOH). After shaking the samples with a vortex for 4 min, 0.5 g of NaC_l and 2 g of anhydrous $MgSO_4$ were added. The tubes were shaken for 1 min up and down by hand. Extracts were centrifuged for 5 min at 3800 rpm and 4ºC. An aliquot of 2 mL supernatant was taken, filtered through a 0.22-mm PTFE filter (Merck Millipore, Billerica, MA, USA), and transferred to a glass vial for HPLC-DAD analysis.

HYDRUS-1D Modeling Description

The HYDRUS-1D model was used to simulate the herbicides' transport in the soil columns. Solute transport in a numerical model is usually described using the relative standard advection and dispersion equation (Šimůnek *et al.*, 2007). The HYDRUS uses the Fickian-based advection- dispersion equations for solute transport. In the case of the solute transport, the governing equation considers advection-dispersion transport in the liquid phase, as well as diffusion in the gaseous phase. While convective transport includes the passive movement of dissolved constituents with water flow, dispersive transport is a result of differential water flow velocities at the pore scale. One-dimensional vertical transfer in variably saturated medium where neither adsorption nor degradation occurs is expressed as follows (Kim *et al.*, 2011):

$$\frac{\partial \theta C}{\partial t} + \rho \frac{\partial s}{\partial t} = \frac{\partial}{\partial z}\left[\theta D \frac{\partial C}{\partial z}\right] - \frac{\partial q C}{\partial z} - \emptyset \qquad (1)$$

where s is the sorbed solute concentration unit (MM^{-1}), θ is the volumetric water content unit (LL^{-3}), C is the solution concentration unit (ML^{-3}), ρ is the soil bulk density unit (ML^{-3}), q is the volumetric water flux density evaluated using the Darcy- Buckingham law unit (LT^{1}), D is the dispersion coefficient accounting for both the molecular diffusion and hydrodynamic dispersion unit ($L2T^{-1}$), and $\emptyset$ is the sink/ source term that accounts for the various zero and first- order or other reactions unit ($ML^{-3}T^{-1}$).

Herbicides Mass Balance in Soil Layer

In the HYDRUS model, the pesticide fate and transport processes affecting the pesticide concentration in the soil layer that are considered are: 1) herbicides' adsorption in soil and subsequent partitioning in soil layer dissolution of applied herbicides, 2) herbicides' transport into and out of the surface soil layer though water percolation, 3) biochemical degradation in the soil layer, and 4) herbicides' desorption from the surface soil layer into water. The mass balance of herbicides in the soil is expressed with Equation (2):

$$\frac{dM_{HSL}}{dt} = M_{PSL-DISS} + M_{HSL-PERC} - M_{HSL-DES} - M_{HSL-DEG} \quad (2)$$

where M_{HSL} is the total mass in the surface soil layer, $M_{HSL\text{-}DISS}$ is the mass of the herbicides' transfer into the surface soil layer herbicides' dissolution process, $M_{HSL\text{-}PERC}$ is the mass of the herbicides' transport in or out of the surface soil layer though percolation, $M_{HSL\text{-}DES}$ is the mass of the herbicides' transfer by the herbicides' desorption process from the surface soil layer into water, and $M_{PSL\text{-}DEG}$ is the mass of the herbicides' dissipation by biochemical degradation in the soil.

Determination of Input Parameters

The input parameters used for predicting the concentration of the herbicides in the leaching columns are reported in Table 1. The determination of the input parameter values was calculated and obtained from either experimental measurement, equations, literature review, the Pesticide Manual and numerical formulas, such as half-life, solubility, Henry's law content, vapor pressure, molecular weight, etc. The parameter values are for the desorption rate constant for atrazine and metolachlor, respectively. However, the dissolution rate constant was calibrated by adjusting the value to fit observations (de Wilde *et al*., 2008).

Results

Leaching Concentration of Atrazine and Metolachlor in Soil Column

The herbicides that are used for weed control and more generally to protect plants usually come into contact with the soil, and their fate and transport processes are affected by a variety of processes (Watanabe, 1993). Understanding pesticide bioavailability is of great importance to environmental regulation and pollution control. The concentrations of atrazine and metolachlor after the application of the herbicides were 2.23±0.04 and 2.15±0.02 mg kg^{-1}, respectively. At the end of the experiment, after the application of the herbicides, the concentrations of atrazine and metolachlor in the soil were as low as 0.0068±0.0015 and 0.0013±0.0012 mg kg^{-1}, respectively, while the concentrations of atrazine and metolachlor in water were 0.14±0.03 and 0.21±0.01 mg kg^{-1}, respectivel. The degradation rate constants ranged from 0.014 to 0.036 day^{-1} for atrazine and from 0.010 to 0.028 day^{-1} for metolachlor. The corresponding half-lives ranged from 23.5 days for atrazine

Table 1. The soil characteristics and pesticides parameters values for HYDRUS-1D model simulation

Parameters	Soil column	
Residual soil water content ($cm^3.cm^{-3}$)	0.1	
Saturated soil water content ($cm^3.cm^{-3}$)	0.37	
Saturated hydraulic conductivity ($cm.min^{-1}$)	0.49	
	Atrazine	**Metolachlor**
Molecular diffusion coefficient ($cm^2.min^{-1}$)	334.73×10^{-6}	305.76×10^{-6}
Adsorption isotherm coefficient ($cm^3.mg^{-1}$)	0.0045	0.003
First-order rate constants (min^{-1})	2.013×10^{-5}	1.944×10^{-5}

and from 24.7 days for metolachlor. The significance of difference in the half-lives was investigated for all combinations for atrazine and metolachlor (Student' s t test, α = 0.05). The results were identical for both herbicides; there was no significant difference. Pesticide degradation was reported to be optimal at the mesophilic temperature range (Topp *et al*., 1997). The half-lives of atrazine and metolachlor computed in this study are in agreement with the literature and a previous field monitoring study.

The HYDRUS-1D Model Simulation

To measure the soil columns in the experiment, stainless steel cylinders with an internal diameter of 5 cm and a length of 35 cm were used to collect agricultural soil at the study area and carried to the laboratory for experimentation, after many days of saturation and drainage.

The soil characteristics and herbicides' parameter values for the HYDRUS-1D model simulation are shown in Table 1. The residual soil water content of both herbicides were 0.1 $cm^3.cm^{-3}$, and the saturated soil water content and saturated hydraulic conductivity were 0.37 $cm^3.cm^{-3}$ and 0.49 $cm.min^{-1}$, respectively.

The results of the simulation are shown in Figure 2; the simulated breakthrough curves (BTCs) of the 2 herbicides with the experimental conditions used the single porosity model to consider equilibrium. At the middle of the soil column, the soil water concentrations increased to maximum values of 2.21 and 2.3 $mg. L^{-1}$ of atrazine and metolachlor at 35 and 32 min and decreased to 0.6 and 0.58 $mg. L^{-1}$, respectively at the final time. The concentration had the same trend with different peak times and the amount of concentration.

The HYDRUS- 1D model simulated the observed herbicide concentrations at 17 cm and 35 cm depths as shown in Figure 3. The maximum observed concentrations of atrazine in the water at the outlet of the soil columns and at 17 cm were about 2.19 and 2.28 mg.L at 35 min, respectively. For metolachlor, the maximum observed concentrations at 35 cm were about 1.24 and 1.18 mg.L, at 120 min, respectively. After the maximum concentrations, the simulated values deviated from the observed values with high accuracy up to the maximum concentrations for both herbicides. The RMSE values between the simulated results and the observed data were about 0.25 for atrazine and 0.26 for metolachlor. On the other hand, the comparison of sorbed concentrations showed different shapes at the final time. The shapes of the simulated values are inverted with the observed data. The simulated sorbed concentration values were higher than the observed data between 4.4 to 5.2 times. Therefore, it seems that more of a calibration effort for the input parameters such as equilibrium partitioning coefficient and dispersity is required for more accurate simulation.

The Comparison of the HYDRUS-1D Simulation and the Experimental Results

The results of the simulation are shown in Figure 3. The HYDRUS-1D model was created and ran with the model parameters shown in Table 2. The main output of this HYDRUS-1D simulation was the concentration of the atrazine and metolachlor which depended on time and depth. According to the result, the concentrations of atrazine and metolachlor were indicated at 0 to 210 min. From the first time, the concentrations of atrazine and metolachlor increased to their maximum values of 0.000596 and 0.000364 mg/cm^3, respectively, at 1 cm of soil depth and decreased to 5.92E-17 and 6.02E-14 mg/cm^{-3}, respectively, at 35 cm of the soil depth of soil columns, while, the peak concentration decreased at the second and final time. At the final time, the concentrations of atrazine and metolachlor had maximum values of 0.00141 and 0.00102 mg/cm^3, respectively, at 4 cm of the soil depth as shown in Figure 4, and the position of the peaks had slightly moved down with the time (Figure 3).

Additionally, the results can compare the simulation results with the analysis data of the soil columns. The comparison showed that the simulation results were similar to and slightly different from the analysis data at 0 to 15 cm but much lower at 15 cm to 35 cm of the soil

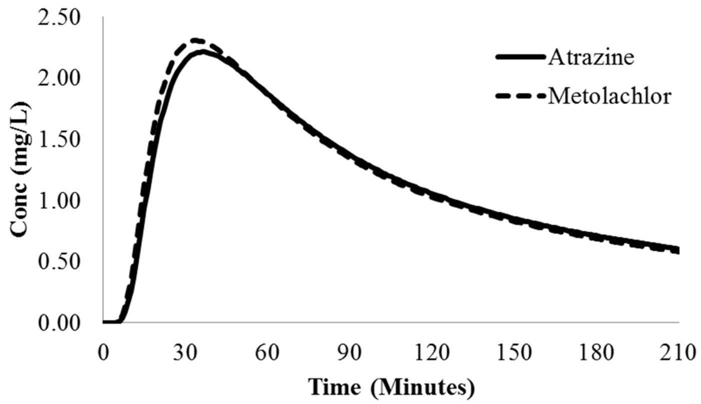

Figure 2. The simulated breakthrough curves (BTCs) of atrazine and metolachlor in water at 0 to 210 min

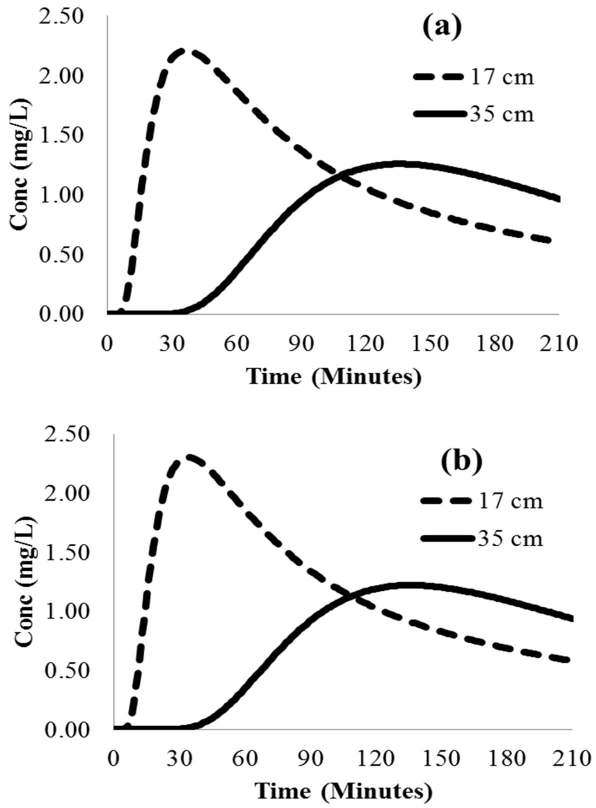

Figure 3. The HYDRUS-1D simulated the observed concentrations of atrazine (a) and metolachlor (b) in water at 0 to 210 min

depth. The R-square and NSI between the simulation result and the analysis data were simulation result and the analysis data were about 0.82 and 0.73, respectively. That proved that the simulation result was satisfactory/ acceptable.

Generally, the simulation values and experimental values are nearly identical; the average relative errors of the simulation values of the soil columns are 3.3% and 5.7%, of atrazine and metolachlor, respectively, and their experimental values are all in the 95% prediction intervals of the HYDRUS-1D model in this study which is more flexible and can accurately predict the herbicides' transport and behavior. The influences of the sorption

Table 2. List of the input parameters used to simulate the HYDRUS-1D model

Name of parameters	Units	Choice and Value
Soil texture	-	Clay Loam
Depth of soil profile	[L]-cm	35
Initial time step	[T]-min	0.01
Minimum time step	[T]-min	0.001
Maximum time step	[T]-min	180
Theta r(θ_r)	-	0.095
Theta s(θ_s)	-	0.41
Alpha	$[L^{-1}]$-cm^{-1}	0.019
The soil water retention function (n)	-	1.31
Ks	$[LT^{-1}]$-cm/min	0.00433333
Number of solutes	-	1
Bulk density	$[ML^{-3}]$-g/cm^3	0.5
Dispersion	[L]-cm	10
Diffuse water	$[L^2T^{-1}]$-cm^2/min	0.2
Kd	$[M^{-1}L^3]$-cm^3/g	3
Sink water	μ_w' $[T^{-1}]$-	0.028
Precipitation	$[LT^{-1}]$-cm/min	0.2
Evaporate	$[LT^{-1}]$-cm/min	0
Number of observation points	-	7

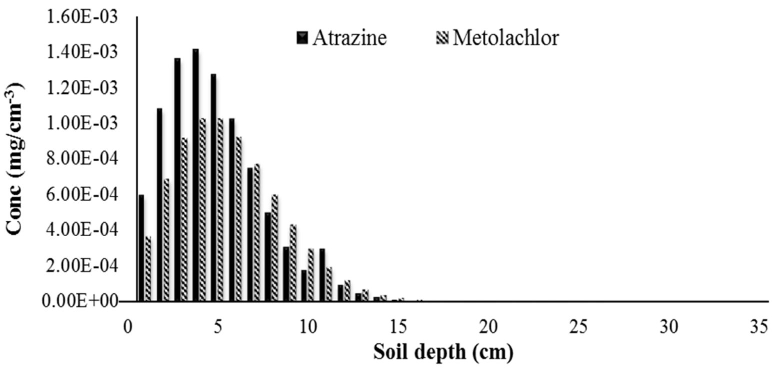

Figure 4. The atrazine and metolachlor concentration by HYDRUS-1D simulation of the soil depth

rate, desorption rate, degradation rate, and diffusion rate on the simulation results in the HYDRUS-1D model were similar to the experimental results. In addition, the simulation results of the herbicides depended on the parameter values of the initial reactant in the HYDRUS-1D model which was used to simulate the migration and transformation mechanisms of the herbicides in the soil columns, and were still identical with the initial reactant.

Discussion and Conclusions

As indicated in Figures 2 and 4, our results suggest that for agricultural topsoil with the texture and/or smaller organic matter content, the corresponding half-lives were 23.5 days and 24.7 days, respectively, of atrazine and metolachlor. The HYDRUS-1D model simulated the concentration of atrazine and metolachlor; the highest concentrations were 0.00141 and 0.00102 mg/cm^{-3} at 4 cm soil depth, respectively, and 5.92E-17 and 6.02E-14 mg/cm^3 at the bottom of soil column at the final time, respectively. The comparison between the simulation results and the analysis data showed big differences in 2 depths of soil at 5 cm and 15 cm, but for both the tendency movements were similar. In addition, the range of the parameters suggested that atrazine and metolachlor were greatly adsorbed in the soil due to boundary interactions and chemical interactions between the herbicides and soil functional groups. The HYDRUS-1D model showed the removal of herbicides at low concentrations and that significant parts of them passed into the adsorbed phase, where the herbicides almost did not decompose. As the herbicides advanced in the columns, the model reproduced the return of the adsorbed and undecomposed atrazine and metolachlor into the liquid phase followed by their removal from the columns. The study also found that the movements of atrazine and metolachlor were highly affected by the soil texture, Kd, and water solubility of the herbicides. Finally, the tendency of pesticide movement depends on the soil water content. However, further calibrations of the transport are still required to improve the data for experiments on the herbicide concentrations in the soil columns.

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References

de Wilde, T., Mertens, J., Spanoghe, P., Ryckeboer, J., Jaeken, P., and Springael, D. (2008). Sorption kinetics and its effects on retention and leaching. Chemosphere, 72(3):509-516.

Ebato, M., Yonebayashi, K., and Kosaki, T. (2001). Predicting Freundlich adsorption of herbicide on soils. J. Pestic. Sci., 30(3):220-231.

Gevao, B., Semple, K.T., and Jones, K.C. (2000). Bound pesticide residues in soil. Environ. Pollut., 108:3-14.

Jaikaew, P., Boulange, J., Thuyet, D.Q., Malhat, F., Ishihara, S., and Watanabe, H. (2015). Potential impacts of seasonal variation on atrazine and metolachlor persistence in andisol soil. Environ. Monit. Assess., 187(12):187-760.

Kim, S.H., Fan, M., Prasher, S.O., Patel, R.M., and Hussain, S.A. (2011). Fate and transport of atrazine in a sandy soil in the presence of antibiotics in poultry manures. Agr. Water Manage., 98(4):653-660.

Organization for Economic Cooperation and Development. (2004). Test No. 312: Leaching in Soil Columns. Organization for Economic Cooperation and Development, Paris, France, 15p.

Raymundo-Raymundo, E., Yu, N.N., Guber, A.K., and Landeros-Sanchez, C. (2011). Adequacy of transport parameters obtained in soil column experiments for selected chemicals. Eurasian Soil Sci., 45:(7):675-683.

Šimůnek, J., Sejna, M., and Genuchten, M.T. (2007). Modeling nonequilibrium flow and transport processes using HYDRUS. Vadose Zone J., 7(2):782-797.

Šimůnek, J., Van Genuchten, M.Th., Šejna, M., Toride, N., Leij, F.J. (1999). The STANMOD Computer Software for Evaluating Solute Transport in Porous Media Using Analytical Solutions of Convection-Dispersion Equation. International Ground Water Modeling Center, Colorado School of Mines, Golden, CO, USA, 32p.

Singh, G., Van Genuchten, M.T., Spencer, W.F., Cliath, M.M., and Yates, S.R. (1994). Measured and predicted transport of two S-triazine herbicides through soil columns. Water Air Soil Poll., 86:137-149.

Topp, E., Vallaeys, T., and Soulas, G. (1997). Pesticides: microbial degradation and effects on microorganisms. In: Modern Soil Microbiology. Van Elsas, J.D., Trevors, J.T., and Wellington, E.M.H., (eds). Marcel Dekker, Inc., NY, USA, p. 547-575

Van Genuchten, M.T. and Wagenet, R.J. (1989). Two-site/ two- region models for pesticide transport and degradation: Theoretical development and analytical solutions. Soil Sci. Soc. Am. J. 53(5):1303-1310.

Van Genuchten, M.T., Šimůnek, J., Leij, F.L., Toride, N., and Šejna, M. (2012). STANMOD: Model use, calibration and validation T. ASABE, 5(4):1353-1366.

Watanabe, T. (1993). Relationship between volatilization srates and physicochemical properties of some pesticides. J. Pestic. Sci., 28:201-209.