

Research Article

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Tribenuron-Methyl Herbicide Decontamination using Vetiver Grass and its Distribution through Soil Profile



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Abstract

Pesticides are powerful toxicants that are easily absorbed, and they tend to accumulate in soil, plants, ground and surface waters. Phytoremediation is an economic and cost efficient technology that uses plants to clean up the environment from pollutants. In this research, the dissipation of tribenuron-methyl in soil profile was investigated. This herbicide was sprayed on the soil surface with doses of 298.57 (C₁) and 597.13 (C₂) kg a.i. ha⁻¹ with vetiver (C₁V and C₂V) and without vetiver (C₁S and C₂S). Additionally, a treatment was considered, in which no herbicide was added to the soil (C₀) to investigate the vetiver growth in normal condition. Soil samples were taken from depths of 0 to 120cm at 10cm increments. Tribenuron-methyl was extracted and measured by HPLC. This herbicide was transported to 90cm of soil surface at the end of the experiment. The tribenuron-methyl removal from soil by vetiver in C₁V and C₂V treatments were 373.7 and 361.6 g ha⁻¹, respectively. In general, at 128 days after herbicide application in all soil profiles, the concentration of tribenuron-methyl in C₁V and C₂V treatments were 39.8% and 30.1% less than C₁S and C₂S treatments, respectively. Concentrations of the herbicide in vetiver treatments did not increased significantly over time at depths of 40-50 and 50-60cm. This demonstrates that not only vetiver grass can absorb this herbicide, but also its root and shoot can stabilize it at shallower soil depths. The mean half-life in all treatments in 0-30cm soil profile was 14.2 days.

Keywords: Herbicide; Soil; Half-life; Dissipation

Introduction

In recent decades, pesticides have been extensively used in agriculture throughout the world. While chemicals used for the protection of crops from weeds and improve yields, they also affect soil and environment health if not properly managed. They are powerful toxicants that are easily absorbed, and they tend to accumulate in soil, plants, foods, ground and surface waters. These effects mainly depend on the active ingredients, application rates, as well as soil properties such as water content, pH and sorptive capacity [1].

Agrochemicals can pose harmful effects to the environment by exposure of the target soil compartment to the atmosphere throughout volatilization, allowing these chemicals to seep into the subsoil and groundwater, even during crop uptake and runoff. The agrochemicals, in spite of their benefits, can pose a threat to environment due to their hazardous side effects [2]. Therefore it is necessary to investigate the influence of pesticides on the natural environment due to their increasing utilization in agricultural practices [1]. Phytoremediation is an economic, cost and energy efficient technology that uses plants to clean up the environment from pollutants [3]. Vetiver

grass (*Vetiveria zizanioides* L.) Nash has shown to be durable in unfavorable environments and has a high level of tolerance to adverse conditions due to its morphological and physical characteristics [4].

Several studies have demonstrated the contaminant-absorbing ability of vetiver grass. Boonsaner et al. [5] conducted an experiment to investigate the bioaccumulation of five organophosphate pesticides in vetiver root and stem. Results showed that the accumulation of compounds increased with their hydrophobicity, with the rate of uptake by vetiver root of methamidophos>ethoprophos>methidathion> methylparathion >EPN. This was opposite of the accumulation pattern seen in the stem. Boonsaner et al. [6] also investigated the absorption of these same pesticides in cabbage and vetiver grass. Results showed that vetiver grass can absorb organophosphate pesticides from agricultural lands. The effects of different concentrations of atrazine and diroun on the growth of vetiver were investigated by Cull et al. [7]. Results indicated that vetiver growth was not affected by the application of atrazine or diuron at concentrations up to 2000µg L⁻¹. Noshadi et al. [8] studied the

concentration of atrazine and its downward movement in the soil profile. Results showed that the concentration of atrazine was reduced in the soil profile up to a depth of 50cm. Murphy et al. [9] investigated the ability of switch grass to degrade atrazine in a phytoremediation setting. Their results demonstrated the capability of switch grass leaf biomass in detoxifying atrazine. Olette et al. [10] studied the toxicity and removal of flazasulfuron, copper, and dimethomorph by four aquatic plants. Results showed that Lemnaminor had the most efficient uptake capacity, followed by Elodea Canadensis and then Cabomba Aquatica. The maximum removal rate of copper, flazasulfuron and dimethomorph was 30, 27 and 11µg⁻¹ fresh weight d⁻¹, respectively.

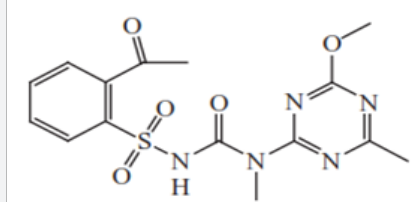


Figure 1: Chemical structures of tribenuron-methyl.

Tribenuron-methyl (TBM), methyl 2-[4-methoxy-6-methyl-1,3,5-triazin-2-yl] carbamoylsulfonate, is a member of the sulfonylurea herbicide family (Figure 1). It kills weeds by inhibiting the synthesis of branched chain amino acids (valine and isoleucine), and thereby stopping cell division and weed growth [11]. Tribenuron methyl (C₁₅H₁₇N₅O₆S) is generally used in agriculture weed control for cereal crops, especially rice. The attention to environmental biodegradation and the fate of sulfonylureas has increased due to their high phyto toxicity to susceptible plants and their possible impact on aquatic ecosystems [12-14]. Tribenuron-methyl is a relatively polar, weak, acidic compound that is soluble in water. It can be extracted with polar organic solvent, buffer solution, or a mixture of organic solvent and water.

The sulfonylureas herbicides are frequently used as pre-emergence and/or post-emergence herbicides at low rates, with typically less than 100g of active ingredient per hectare [15-17]. They have low acute and chronic mammalian toxicities [16,18]. However, due to their polar nature, fairly high water solubility, high phyto toxicity and adverse impacts on mammals, they are considered potential water pollutants and present environmental risks for crops, aquatic plants and microorganisms [19-22]. Therefore, it is necessary to evaluate the dissipation of these herbicides and determine their terminal residues.

Similar to other sulfonylureas, both microbial degradation and chemical hydrolysis play important roles in the degradation of tribenuron-methyl. The cleavage of the sulfonylurea bridge is the major initial chemical reaction for sulfoneas degradation, especially under acidic conditions (Berger et al. 1998). However, microbial degradation of sulfonylureas is more effective and

rapid than the chemical hydrolysis process, either by complete mineralization [23,24] or co-metabolism [14,25,26]. Aqueous solubility of tribenuron-methyl increases with an increase in pH, ranging from 50.0mgL⁻¹ at pH 5 to 18.3gL⁻¹ at pH 9. Some researchers have focused on residue of tribenuron-methyl in soil [27,28], water [29], agriculture products [30] and fruits [31]. Riise [32] demonstrated that the extent of tribenuron-methyl sorption to soil increases with decreasing pH, especially for silt and clay soils. The extent of tribenuron-methyl sorption was higher in silt and clay fractions. Tribenuron methyl has a pka value of 4.7 and was relatively stable with K_{ow} ~4 in pH range of 4-11. The low K_{ow}-value indicates that hydrophobic sorption of this herbicide is not of much importance. The physico-chemical characteristics of pure tribenuron-methyl herbicide are shown in Table 1.

Table 1: Physico-chemical properties of pure Tribenuron-methyl.

Parameter	Values and Conditions
	Tribenuron-methyl
Vapor Pressure	5.2* 10 ⁻⁸ Pa at 25 °C (extrapolated)
Melting point	142±1.1 °C
Boiling point	Not application
Temperature of decomposition	in the range 145 to 185 °C
Solubility in water	0.0489g/l at 20 °C at pH 5
	2.04g/l at 20 °C at pH 7
	18.3g/l at 20 °C at pH 9
Octanol/water partition coefficient	Log P _{ow} = 2.6 at 25 °C at pH 5
	Log P _{ow} = 0.78 at 25 °C at pH 7
	Log P _{ow} = 0.30 at 25 °C at pH 9
Hydrolysis Characteristics	<1 day at 25 °C at pH 5
	<15.8 days at pH 7
	Stable at 25 °C at pH 9

There is little information about phytoremediation and soil residue of tribenuron-methyl herbicides, despite being one of the most common herbicide used in many countries. This research investigates the role of vetiver grass in reducing the concentration of this herbicide and its distribution in soil profile.

Materials and Methods

Table 2: Soil Physical Characteristics.

Soil Depth (cm)	Soil Depth (cm)	*PWP (cm ³ /cm ³)	ρb *(g/cm ³)	Soil Texture
0-30	0.27	0.12	1.23	silty clay loam
30-60	0.31	0.16	1.46	silty loam
60-90	0.31	0.16	1.46	silty loam
90-120	0.31	0.16	1.46	silty loam

*pb: bulk density; PWP: permanent wilting point; FC: Field capacity

This research was conducted in a greenhouse in the college of agriculture at Shiraz University in Shiraz, Iran. Fifteen lysimeters were constructed from PVC pipes with a height of 120cm and diameter of 40cm. The lysimeters were filled with field soil which was collected from the depths of 0-30, 30-60, 60-90, 90-120cm, separately. They were collected separately to simulate real field conditions. Some physical characteristics of soil are shown in Table 2.

All vetiver grass shoots were cut to have identical heights of 30cm and were then planted in 9 lysimeter on July 11th. After one week, during which the crops were properly settled, tribenuron-methyl was applied to the soil and was followed by 3 replications. To investigate the dissipation of tribenuron-methyl in vetiver and soil, this herbicide was dissolved in water and sprayed on the soil's surface. Tribenuron-methyl doses were 298.57 (C₁) and 597.13 (C₂) kg.ha⁻¹ with three replications in a completely randomized design. The applied herbicide concentrations were much higher than the recommended dosage.

Table 3: Description of different treatments.

Treatment	Concentration (mg/kg soil)	Description
	Tribenuron-methyl	
C ₀ V	0	with vetiver and no herbicides
C ₁ V	484.6	with vetiver and herbicide in C ₁ concentration
C ₂ V	969.2	with vetiver and herbicide in C ₂ concentration
C ₁ S	484.6	no vetiver with herbicide in C ₁ concentration
C ₂ S	969.2	no vetiver with herbicide in C ₂ concentration

The vetiver grasses were collected at 128 days after spray application, the soil samples were collected from different depths, in 40, 62, 83, 104 and 128 days after herbicide application. For each treatment of vetiver crop (CV), a treatment with no plant (CS) was designated as a control in order to monitor the herbicide concentrations throughout the soil profile. Additionally, treatment with 3 replications was considered, in which no herbicide was added to the soil and crop (C0).The treatments are shown in Table 3.

Table 4: Depths and dates of soil sampling.

Date	Soil Depth* (cm)
20-Aug-11	0-70
10-Sep-11	0-70
1-Oct-11	0-70
22-Oct-11	0-70
15-Nov-11	0-120
*Soil samples from 0 to 70cm and 0-120cm were taken with 10cm increments	

Three TDR probes were placed in all CV treatments at soil depths of 15, 45, 60cm to measure soil water content. Irrigation was scheduled based on the soil water deficit according to field capacity. Forty days after herbicide application, soil samples were taken from depths of 0 to 70cm at 10cm increments. Since the herbicide may have been transport to the deeper depths of the soil profile, at the end of growing season, sampling was done down to 120cm of the soil surface (Table 4). On November 15th, (at 128 days after herbicide application), the crops were removed from the soil and both roots and shoots were separated and air-dried in the laboratory. These were kept in -20 °C until they were moved to the laboratory for herbicide's residual concentration analysis Table 4.

Extraction method [40]

Sieved and air-dried soil samples (10g) and 10mL twice distilled water were mixed and was shaken for 5 minutes. A volume of 6mL acetonitrile was added to the samples, which were then shaken for 45 minutes. The 0.75g NaCl was added to the samples and shaken for 1 minute. The samples were then centrifuged at 1500rpm for 10 minutes to form a three-layer parathion. Then 3mL of the supernatant layer were removed and transferred to another tube and 75mg PSA salt and 360g MgSO₄ was added. Then the samples were shaken for 1 minute and centrifuged for 10 minutes. They were then passed through the 45 micrometers filter and injected into the HPLC. The column, flow rate and UV wave length of HPLC were C-18 (25m length), 0.7ml/ min, 30 °C and 230nm, respectively. Carrier phase were water and acetonitrile at a ratio of 4 to 6±1.0 percent phosphoric acid.

The stock solution of tribenuron-methyl containing 1000mgL⁻¹ was obtained from agricultural ministry of Iran. From this stock solution, 20, 10, 5, 2.5, 1.0, 0.5, 0.1mgL⁻¹ were prepared by serial dilution. Each solution with three replications was injected into HPLC.

Results and Discussion

Table 5: The accumulated tribenuron-methyl herbicide in roots and shoots of the vetiver grass.

Treatment	Concentration (µg/kg)	Description
C1V	22	roots
	96	shoots
Total	118	
C2V	11	root
	154	shoot
Total	165	

The concentrations of accumulated tribenuron-methyl herbicide in the roots and shoots of the vetiver grass are shown in Table 5. The total concentration of tribenuron-methyl accumulated in vetiver in C₁V and C₂V treatments were 118 and 165µgKg⁻¹, which were 25 and 40 percent of total amount of herbicide residue in soil, respectively. The accumulated of

tribenuron-methyl in vetiver shoot of C₁V and C₂V treatments was 336.4% and 130% more than vetiver roots, respectively (Table 5). Therefore, these results demonstrate that herbicides uptake by vetiver grass increases with an increase in herbicide application. The maximum accumulation was seen in the vetiver shoots Table 5.

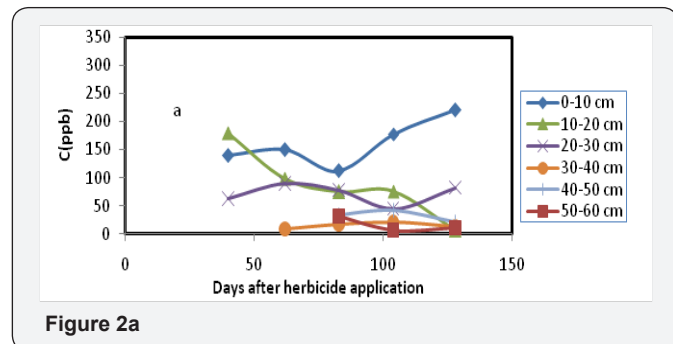


Table 6: Dried weight of vetiver shoots and roots.

Treatments	Dried Weight (Kg ha ⁻¹)		Total
C ₀ V	12024.7 a	3468.9 a*	15439.6 a
C ₁ V	3453.0 b	1920.4 b	5373.4 b
C ₂ V	2250.8 c	1365.4 c	3616.2 c

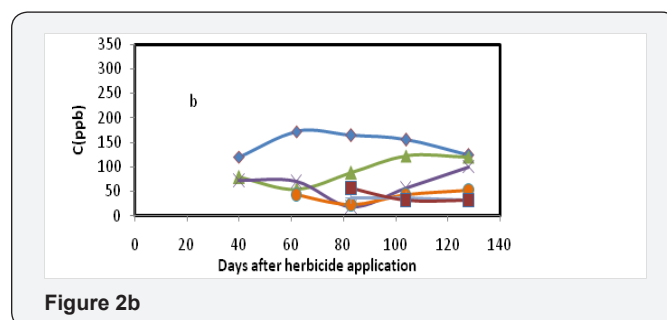
*Means followed by the same letters in columns are not significantly different at 5% level of probability, using Duncan multiple test.

Dried weight of vetiver shoots and roots for all treatments are shown in Table 6. Dried weight of vetiver shoots in C₁V and C₂V treatments decreased 70 and 81% compared to C₀ treatment. Additionally, dried weight of vetiver roots in C₁V and C₂V were decreased by approximately 44 and 60%, compared to the control treatment (C₀). Some leaves of the vetiver shoots in C₁V and C₂V treatments were dried during the experiment but the vetiver grass tolerated high levels of vetiver concentrations. There was also a significant difference among the dried weights of vetiver shoots and roots in different treatments at 5% level of probability, using Duncan multiple test. According to Table 5 & 6, the tribenuron-methyl removal from soil by vetiver in C₁V and C₂V treatments were 373.7 and 361.6g/ha⁻¹, respectively.

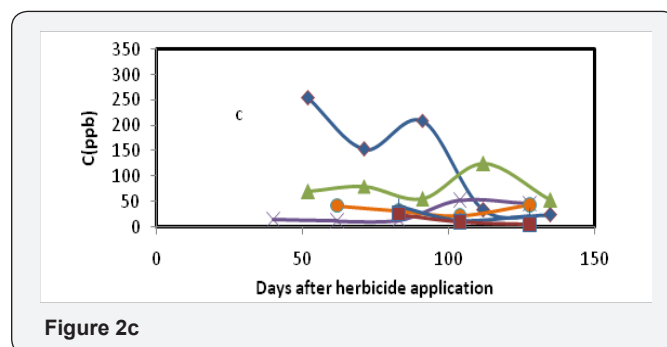
Concentration of tribenuron-methyl in the soil profile at different depths and dates after herbicide application is shown in Figure 2a. It illustrates that this herbicide transport downwards to 90cm of soil surface at the end of the experiment.

In C₁V treatment at 40, 62, 83, 104 and 128 days after tribenuron-methyl application, the concentration in 0-10cm soil depth was 80, 150, 112, 178 and 139 μg Kg⁻¹ soil, respectively. On the same dates, the concentration of tribenuron-methyl in 10-20cm soil depth was 180, 93, 75, 76 and 70 μg Kg⁻¹ soil, respectively. Therefore, in 0-10cm soil depth, at 62, 83, 104 and 128 days after this herbicide application, soil residues were 87.5, 40.0, 122.5 and 73.8% higher than 40 days after application, respectively. But in 10-20cm soil depth at 40 days after application, soil residues were 93.5, 140.0, 136.8 and 247.1% higher than 62, 83, 104 and 128 days after application, respectively. In other word in some

soil depths, especially 0-10cm, the concentration of herbicides was increased in some days after application. This might have happened due to upward flow of water in soil which transports the herbicides to upper soil layers otherwise known as the wick effect. The cracks in soil surface maybe another reason, because these cracks in soil surface cause lack of sufficient contact time for washing herbicide in to the lower soil layers, which decrease leaching efficiency. The volatilization of herbicide in deeper soil layers could be another reason of upward flow of herbicide in the soil profile. Upward movement of tribenuron-methyl to the surface of soil can be seen in Figure 2b.



In C₂V treatment at 40, 62 and 83 days after herbicide application, the tribenuron-methyl concentration at 0-10cm soil depth was 228.9, 88.9 and 275.0% higher than 10-20cm soil depth, respectively, due to upward movement of herbicide. But at 104 and 128 days after herbicide application, the tribenuron-methyl concentration at 0-10cm soil depth was 74.5 and 53.8 % less than 10-20cm soil depth, respectively, due to leaching process.



At 128 days after herbicide application of C₁V treatment, the concentration of tribenuron-methyl at 0-10 and 10-20cm decreased to 21.9 and 90.8%, relative to 104 days after application, respectively. However, it increased to 82.2% at 20-30cm, which is mostly due to the leaching process. The concentration of tribenuron-methyl in C₁S treatment at 62 days after application and 0-10cm soil depth was 43.3% higher than 40 days after application. However, on the same date, the concentration of tribenuron-methyl at 10-20cm soil depth was 30.8% less than 40 days after application. This can be attributed to upward movement of the herbicide. Tribenuron-methyl herbicide leached down gradually with time to depths of 80cm. A similar trend was observed for C₂V and C₂S treatments (Figure 2c & 2d).

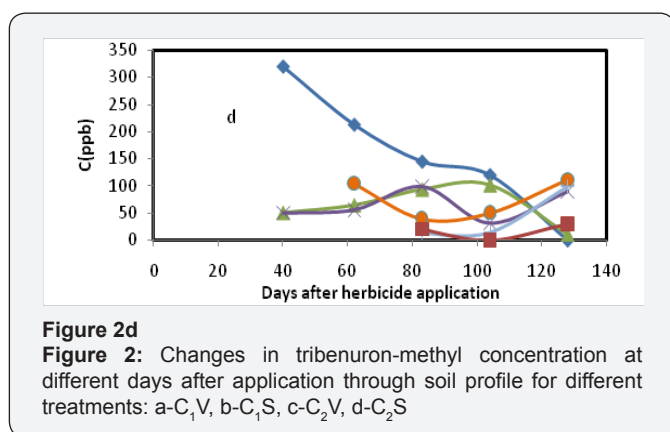


Figure 2d
Figure 2: Changes in tribenuron-methyl concentration at different days after application through soil profile for different treatments: a-C₁V, b-C₁S, c-C₂V, d-C₂S

At 128 days following herbicide application, concentrations of tribenuron-methyl for C₁V treatment in 10-20, 20-30, 30-40, 40-50 and 50-60cm soil depths were 94, 43, 75, 66 and 66% less than C₁S treatment. At depths of 0-10 and 70-80cm, herbicide concentrations in C₁V treatment were 12 and 33 % more than C₁S treatment, respectively. At depth of 80-90cm, it was 0µgkg⁻¹ soil in C₁S treatment which reached to 5µgkg⁻¹ soil in C₁V treatment.

At 128 days after herbicide application the concentration of tribenuron-methyl in C₂V treatment in 20-30, 30-40, 50-60 and 60-70cm soil depths were 47, 60, 76 and 62 % less than C₂S treatment, respectively. In this date, the tribenuron-methyl concentrations at 0-10, 10-20, 70-80cm soil depths for C₂S treatment were 0, 11 and 0µgkg⁻¹soil, respectively and for C₂V treatment were 24, 52 and 36 µgKg⁻¹ soil, respectively.

In general, at 128 days after herbicide application in all soil profiles (0-90cm soil depths), the concentration of tribenuron-methyl in C₁V and C₂V treatments were 39.8% and 30.1% less than C₁S and C₂S treatments, respectively.

Comparing Figure 2a & 2b, the concentration of tribenuron-methyl in 0-10cm soil depths is seen to decrease over time with a steeper slope in C₂S than C₂V, which represents the transport of herbicides down to lower depths in absence of vetiver grass. An increase of concentration in the last three samples (at 83, 104 and 128 days after application) in 30-40, 40-50, 50-60cm soil depths confirms this.

Concentrations of the herbicide did not increase significantly over time at depths of 40-50 and 50-60cm. This demonstrates that not only can vetiver grass absorb this herbicide, but also its roots can stabilize it at shallower soil depths.

Dissipation equations

Terrestrial field dissipation (TFD) half-life is a physical-chemical property of pesticides. The half-life of a substance is defined as the time it takes for a substance to decrease its amount by half. For all the samples, exponential relationships were applied between soil residue concentrations against time, corresponding to first order rate equation.

$$C_t = C_0 e^{-kt} \quad (1)$$

Where:

C= Value of herbicide at time, mg.ha⁻¹

C₀ = initial amount of herbicide, mg.ha⁻¹

k = dissipation rate constant, days⁻¹

t = time, days

The half-life (t_{1/2}) was determined from the k value for each experiment, as calculated by Wang et al. [33] and Liang et al. [34].

$$t_{1/2} = \ln 0.5/k \quad (2)$$

Where

t_{1/2} = half-life of herbicide, days

k = dissipation rate constant,

Table 7: Dissipation equation and half-lives of tribenuron-methyl in soil profile.

Treatment	Soil Depth (cm)	Dissipation Equation	R ²	Half-lives (d)
C ₁ V	0-10	C=11866 e ^{-0.04t}	0.49	17.3
	20-Oct	C=18249 e ^{-0.06t}	0.8	11.6
	20-30	C=5580 e ^{-0.04t}	0.543	17.3
C ₁ S	0-10	C=15062 e ^{-0.04t}	0.55	17.3
	20-Oct	C=6333 e ^{-0.04t}	0.47	17.3
	20-30	C=4613 e ^{-0.04t}	0.48	17.3
C ₂ V	0-10	C=48129 e ^{-0.07t}	0.77	9.9
	20-Oct	C=19203 e ^{-0.05t}	0.64	13.9
	20-30	C=3384 e ^{-0.05t}	0.4	13.9
C ₂ S	0-10	C=16182 e ^{-0.08t}	0.76	8.7
	20-Oct	C=18466 e ^{-0.06t}	0.68	11.6
	20-30	C=9173 e ^{-0.05t}	0.55	13.9

The mean half-lives of tribenuron-methyl in C₁V, C₁S, C₂V and C₂S treatments in 0-30cm soil profile were 15.4, 17.3, and 12.6 and 11.4 days (Table 7). Dong et al. [35,36] demonstrated that the half-life of tribenuron-methyl was 1.27-5.37d Table 7.

Conclusion

Agrochemicals can pose harmful effects to the environment by exposing the target soil compartment to the atmosphere throughout volatilization, transporting down to subsoil and groundwater, crop uptake and runoff [37-39]. In this research, the ability of vetiver grass to reduce the concentration of tribenuron-methyl, in soil and its distribution through the soil profile was investigated.

The vetiver was able to tolerate very high concentrations of tribenuron-methyl. The results demonstrated that vetiver grass is capable of reducing the herbicide concentration in soil [40].

The total concentration of tribenuron-methyl accumulated in vetiver in C₁V and C₂V treatments were 25 and 40 percent of total amount of herbicide residue in soil, respectively. These herbicides accumulated in vetiver shoots more than its roots. Results showed that not only does vetiver grass can uptake these two herbicides, but its root can stabilize them in upper soil depths.

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