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Dissipation dynamics of terbuthylazine in soil during the maize growing season

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Ever since terbuthylazine (TBA) replaced atrazine in herbicide crop treatment, its much greater persistence has raised considerable environmental concern. The aim of our field experiment was to establish the dissipation dynamics of TBA and its degradation product desethylterbuthylazine (DET) in soil over five months of maize growth. We applied TBA as part of pre-emergent treatment in the regular and double-the-regular amounts. Soil samples were collected periodically at the following depths: 0-10 cm, 10-20 cm, 20-30 cm, and 30-50 cm. For TBA and DET soil residue analysis we used microwave-assisted extraction with methanol, followed by HPLC-UV/DAD. Regardless of the application rate, more than 80 % of the applied TBA dissipated from the first 50 cm of soil in the two months after herbicide application and 120 mm of rainfall. Three months later (at maize harvest), less than 4 % of total TBA remained in the soil, mostly in the top 20 cm rich with organic carbon on which TBA is likelier to adsorb. The loss of TBA from soil coincided with the rise in DET, especially the top soil layers, during the periods of low rainfall and highest soil temperatures. This points to biodegradation as the main route of TBA dissipation in humic soils. The applied amount had no significant effect on TBA dissipation in the top (humic) layers, but in the layers with less than 1 % of organic carbon, it was higher when the double-the-regular dose was applied.

KEY WORDS: *application rate; desethylterbuthylazine; field conditions; herbicide; persistence*

Terbuthylazine (6-chloro-*N*^{2}-ethyl-*N*^{4}-*tert*-butyl-1,3,5-triazine-2,4-diamine, TBA) is a broad-spectrum chlorotriazine herbicide used in pre- and post-emergent treatment of a variety of agricultural crops as well as roads, railways, and industrial area (1). Although introduced in 1967, TBA application in agriculture has intensified since 2007 with the ban of atrazine in the European Union (2, 3). In comparison with atrazine, TBA is less soluble in water, more hydrophobic, and more resistant to biodegradation in soil. Judging by several studies (4-6), TBA also seems to dissipate in agricultural soil at a much lower rate than atrazine. By definition, dissipation is a sum of all possible outcomes of the parent herbicide, including volatility, plant absorption, leaching, runoff, and chemical or microbial degradation (transformation) (7-10). All of the above means that it is more persistent in the environment. This has been confirmed by our previous comparative field study (11), when TBA residues were measurable in the uppermost 60 cm of soils even after 17 months of application, while atrazine residues persisted only 5 months. Because of this persistence, TBA may present a similar environmental concern as atrazine (4, 12).

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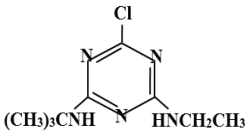
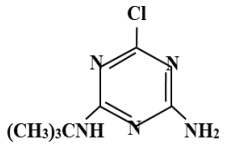
If a herbicide is applied before crop emergence (as in this study), then only a very small portion will be absorbed by plant roots (13), while the bulk will remain in soil from where it can enter surrounding waters (10, 14-16). The mobility of a herbicide (leaching/runoff) or its retention in soil (adsorption) is highly affected by its characteristics (such as hydrophobicity), soil type (pH and humus/clay content), and soil moisture level and temperature (6, 8, 17). Higher soil temperatures favour herbicide desorption and degradation (18, 19). Considering that the hydrolysis of TBA in aqueous soil suspension at pH 7-9 is very slow (half-life >200 days), its transformation in soil generally derives from biodegradation (20). Agricultural soil microorganisms can degrade TBA into an *N*-deethylated product 6-chloro-*N*-*tert*-butyl-1,3,5-triazine-2,4-diamine or, shortly, desethylterbuthylazine (DET) (21, 22). Table 1 shows the structures and properties of TBA and DET relevant for their dissipation in soil (23).

Numerous distribution models have been developed to assess herbicide water contamination potential based on standard field scenarios, but to produce accurate assessments, all of them require sufficient data from field dissipation studies in agricultural soil under the most common conditions (6, 9, 24-26). The objective of this field study was therefore to provide these data about the vertical distribution of TBA and DET in the uppermost 50 cm of

Table 1 *Physicochemical properties of terbuthylazine and desethylterbuthylazine (23)*

Property (at 20-25 °C; pH 7)	Terbuthylazine TBA	Desethylterbuthylazine DET
		
Solubility in water (mg L^{-1})	8.5	327.1
log K_{ow} [a]	3.4	2.3
pK_a [b]	1.9	-
Vapour pressure (mPa)	0.12	0.35
Aqueous hydrolysis pH 7-9	Stable	Stable
K_f [c] (mL g^{-1})	2.1-10.5	0.3-3.3

[a]*octanol-water partition coefficient (hydrophobicity);* [b]*dissociation constant;* [c]*Freundlich coefficient (soil adsorption intensity); - no data*

silty clay loam soil under relatively humid and warm field conditions during a maize growing season.

MATERIALS AND METHODS

Chemicals

Analytical-grade TBA (CAS No. 5915-41-3; 99.1 %) and DET (CAS No. 30125-63-4; 99.8 %) were supplied by Riedel-de Haën (Seelze, Germany). For the field experiment we applied the commercial TBA formulation Radazin TZ-50+ (50 % of active ingredient, Herbos, Sisak, Croatia). All solvents were of analytical grade and supplied either by Merck (Darmstadt, Germany) or Kemika (Zagreb, Croatia). Stock and working standard solutions were prepared in acetonitrile and ultrapure water (Millipore, Bedford, MA, USA), respectively.

Field study

The field study took place at the experimental location Šašinovec (20 km northeast of Zagreb). The soil received a conventional pre-sowing treatment (mineral fertilisation and tillage) and the maize (*Zea mays* L.) was sown in May. The area was divided into twelve 65-square-meter plots arranged in a randomised block design. Eight plots received pre-emergent TBA treatment, four at the regular rate (1.5 kg active ingredient/ha; 1R) and four at double-the-regular rate (3.0 kg active ingredient/ha; 2R). The remaining four plots served as untreated controls. The field had not been treated with TBA before, which was verified by an analysis of its residues in control soil samples.

Soil samples were taken from each plot using a gouge auger at the following depths (layers): 0-10 cm, 10-20 cm, 20-30 cm, and 30-50 cm on days 7, 19, 29, 40, 52, 62, 71, 88, 112, 134, and 155 of the experiment. To achieve representative samples, the soil from each plot and from each layer was sampled in triplicate and combined into one composite sample for residue analysis. Wet soil samples were deep-frozen until analysis. Soil water content was determined immediately, before the extraction of residues, by weighing the soil portion before and after air-drying at 110 °C until constant was reached mass (which took several hours). Air-dried samples were characterised for soil properties by layer (Table 2). Rainfall and average air/soil temperatures were monitored daily during the maize growing season (May to October; Table 3).

Analytical procedure

TBA and DET residues were extracted from wet soil with methanol in the soil-to-solvent ratio of 1:5 using microwave-assisted extraction (MARS X, CEM, Matthews, NC, USA) at 110 °C (10 min) with medium magnetic stirring. When cooled to room temperature, the extract was centrifuged, the supernatant decanted, and the organic solvent evaporated under a nitrogen stream. The water

Table 2 *Characteristics of agricultural soil layers*

Layer depth* (cm)	Clay (%)	Silt (%)	Sand (%)	Humus (%)	C_{org} (%)	pH_{water}
0-10	34.0	63.2	2.8	3.4	1.6	7.5
10-20	34.6	62.3	3.1	3.2	1.5	7.7
20-30	33.6	63.2	3.2	1.7	1.1	7.8
30-50	32.7	64.8	2.5	1.6	0.9	7.9

** textural class of all soil layers: silt clay loam; C_{org} = soil organic carbon content*

Table 3 *Meteorological conditions during maize growing season*

Month	Total rainfall* (mm)	Average air temperature (°C)	Average soil temperature (°C)			
			0-10 cm	10-20 cm	20-30 cm	30-50 cm
May	61	14±3	16±2	17±2	17±1	17±1
Jun	29	20±5	23±5	23±5	22±4	21±4
Jul	33	23±3	28±2	27±2	26±2	25±2
Aug	151	23±4	26±5	26±4	26±3	25±3
Sep	127	16±3	18±3	19±3	19±2	20±2
Oct	33	13±3	13±2	14±2	14±1	15±1

**the cumulative rainfall from May to October was 434 mm*

residue was diluted with distilled water to the volume of 1.0 mL and filtered through a 0.2 µm PTFE filter (Waters, Milford, MA, USA). Mass concentrations of the TBA and DET extracts were determined using a liquid chromatograph Varian ProStar with a diode-array detector (UV DAD) adjusted to 213 and 221 nm (Varian, Walnut Creek, CA, USA) and a 250 mm capillary XBridge C_{18} column with internal diameter of 4.6 mm and 5 µm particle size (Waters, Milford, MA, USA). The injected sample volume was 0.1 mL, the mobile phase for gradient elution consisted of acetonitrile and 0.01 mol L^{-1} phosphate buffer (pH 7), and the flow rate was 1 mL min^{-1}. We followed the procedure as described in detail in our earlier study (11). The average recoveries were 83±6 % for TBA and 93±5 % for DET. The detection limits for both compounds were 0.2 ng g^{-1}. The results were recalculated based on soil dry mass (d.m.).

Dissipation kinetics

The persistence of TBA in soil under field conditions was calculated following the single first-order kinetics model:

$$w_t = w_0 \exp(-k_1 t)$$

where w_t (ng g^{-1}) represents the TBA mass fractions determined in soil at time t (d), w_0 is the initial TBA mass fraction, and k_1 is the dissipation rate constant (d^{-1}) (27). Plots of model-predicting curves were obtained using nonlinear least squares regression. The time needed for 50 % of the initial herbicide mass fraction loss (DT_{50}) was calculated according to the relation: $DT_{50} = \ln(2)/k_1$.

The effect of soil properties and TBA application rate on DT_{50} was tested with correlation matrices. All statistical analyses were performed using Statistica 10.0 (StatSoft Inc., Tulsa, OK, USA), and the significance threshold was set at $P<0.05$.

RESULTS AND DISCUSSION

Field conditions during monitoring

Pedological analysis showed that the main difference between the sampled soil layers was in the humic (organic carbon) content. The surface layers (<20 cm of depth) were quite humic and slightly alkaline, while the deeper layers (20-50 cm) were mostly mineral and moderately alkaline. The two did not significantly differ in texture.

Over the five months of monitoring, the field was exposed to moderate precipitation (434 mm in total). Cumulative rainfall per month ranged between 29 mm and 151 mm, the latter recorded in August, three months after TBA application. The first rainfall (50 mm) came only four days after TBA application, which may have accelerated TBA dissipation through leaching and runoff.

In May, when TBA was first applied, air and soil temperature averages were 14 °C and 16 °C, respectively and did not favour TBA evaporation from topsoil. In the same month, soil temperatures differed 1-3 °C between the layers. The highest soil temperatures and the lowest soil moisture content (13-18 %) were recorded in July and August. These conditions favour greater TBA biodegradation. During the other months of monitoring, soil moisture content ranged between 22 % and 28 %. Moisture content this high probably enhanced the adsorption of TBA in the upper, more humic and alkaline layers, which decreased its availability for dissipation.

TBA and DET distribution in soil after regular and double-the-regular TBA application

Figure 1 shows the levels of TBA and DET by soil layers after regular TBA application (1R). One week after the application, only 44 % of TBA was recovered from soil while the rest was dissipated, most of which quite likely with the first heavy rainfall and by biodegradation into DET.

TBA levels in the top 10 cm of soil decreased exponentially until July, after which dissipation slowed down. The 10-20 and 20-30 cm layers had the highest levels of TBA residues in the first 40 days after TBA application, which coincides with 90 mm of rainfall and gradual air/soil temperature rise for 6 °C. Further air/soil temperature rise for 5 °C and poor rainfall until mid August may explain a slight shift of TBA residues to the topsoil (see monitoring records for days 52 and 88 in Figure 1), possibly due to capillary mobility. Late August and September were characterised by a few showers (totalling 260 mm of rain), which may explain the drop in the topsoil (0-10 cm) and the two sublayers (10-30 cm) to less than 2 % and 1 % of

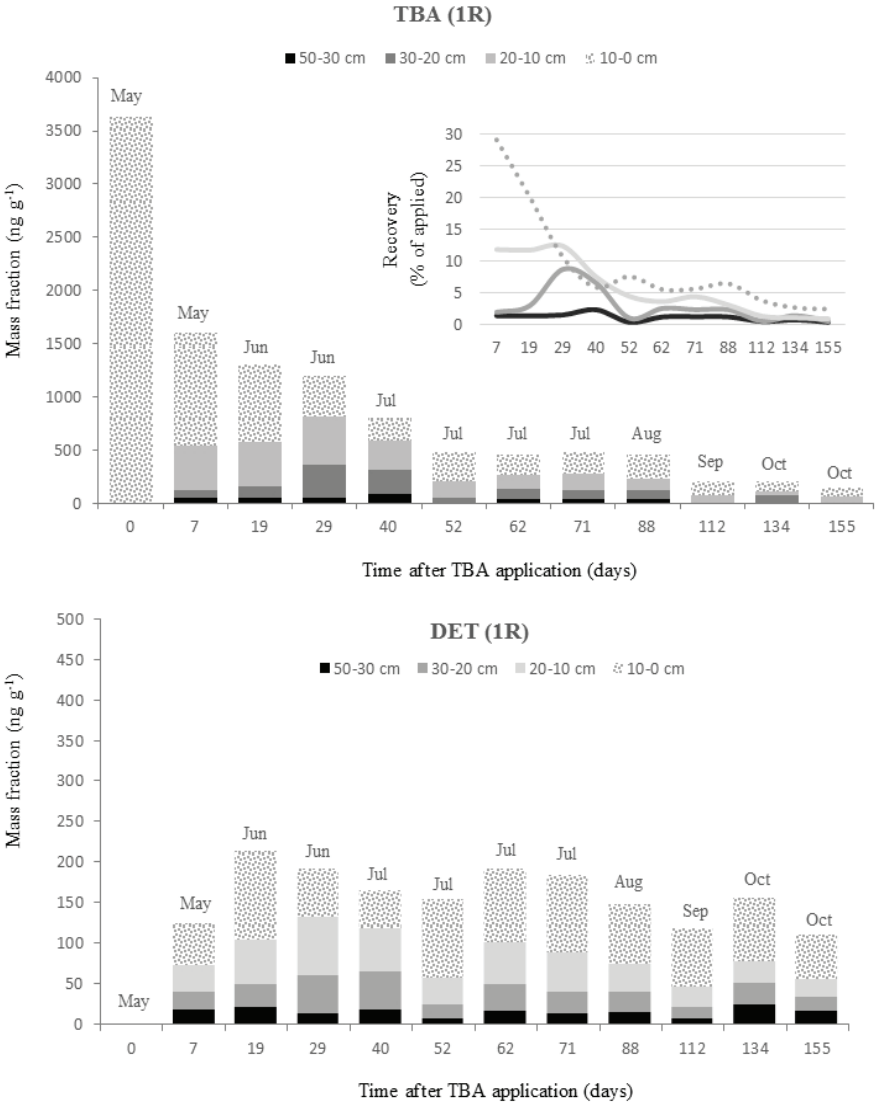


Figure 1 *Distribution of terbuthylazine (TBA) and its metabolite desethylterbuthylazine (DET) in soil after regular TBA application (1R) during maize growing season*

the applied TBA, respectively until the end of monitoring in October.

In the bottommost layer (30-50 cm) TBA levels never exceeded 3 % of its applied amount. Biodegradation of TBA into DET in slightly alkaline soil rich in humus is a significant route of TBA dissipation in soil, especially in the warm months. Our earlier microcosm study of TBA degradation in native and sterilised agricultural silty clay loam soil showed a negligible effect of hydrolysis on TBA dissipation (11). DET was found in all soil samples, but the upper layers had two to four times higher levels than the deeper layers. The highest DET levels (4.5 % of the applied TBA) were recorded on day 19 after TBA application, and kept dropping in the upper layers by the middle of the summer, when warm weather could enhance biodegradation. DET is more polar and therefore more mobile than its parent molecule, which is why its levels in all samples never exceeded 5 % of the applied TBA. In the deeper layers (20-50 cm) it shifted between 0.5 % and 1.8 % of the applied TBA.

Figure 2 shows that when we applied twice as much TBA, TBA and DET levels retained similar distribution patterns across the layers and over time. Seven days after application more than 60 % of the applied TBA dissipated. In the topsoil (0-10 cm) TBA continued to decline exponentially, except for a temporary rise in mid July, to less than 2 % of the applied dose in October. Its levels in deeper layers increased as long as the soil moisture was above 20 % (until day 52) and were the highest after 90 mm of cumulative rainfall (between days 29 and 40). In October, they stayed below 3 % of the applied TBA.

DET levels, in contrast, were significantly higher in the upper, humus-rich layers, than in the deeper layers probably due to biodegradation, but the absolute amounts were lower when the double dose was applied. This points to the greater pollution potential of TBA for the environment, water in particular, if it is applied in high amounts.

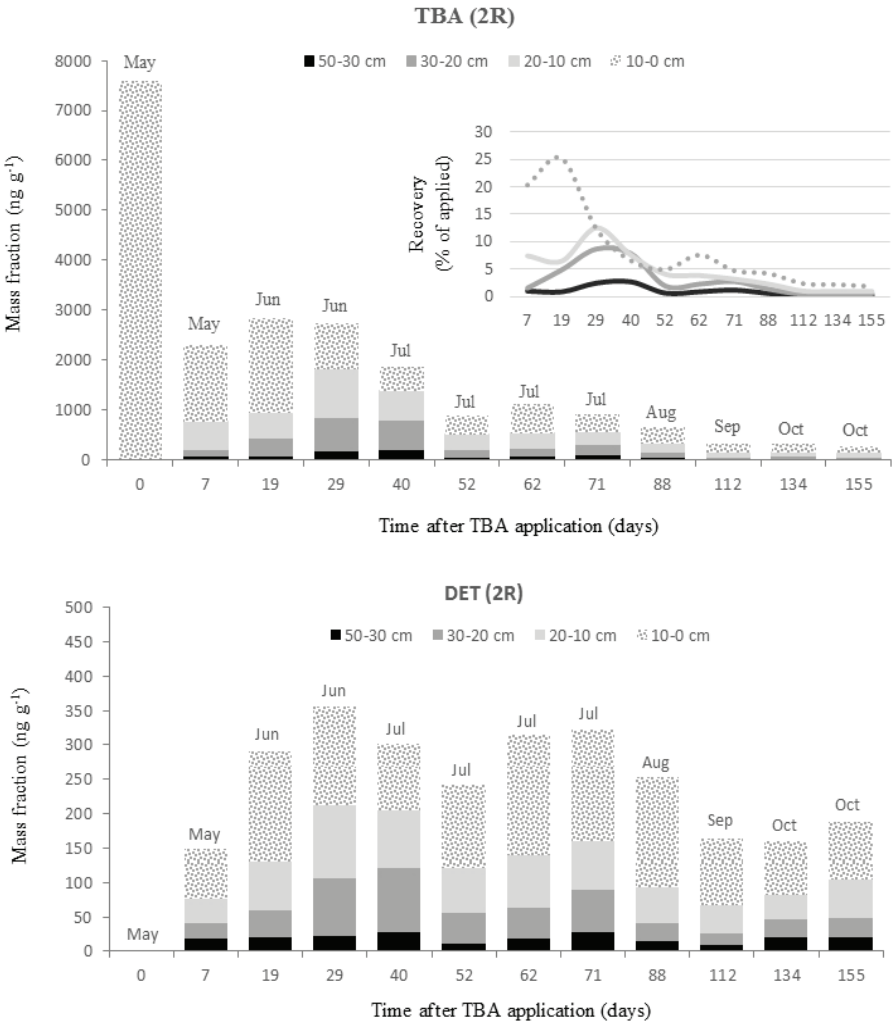


Figure 2 *Distribution of terbuthylazine (TBA) and its metabolite desethylterbuthylazine (DET) in soil after double-the-regular TBA application (2R) during maize growing season*

TBA persistence in soil layers at different application rate

Table 4 shows the persistence of TBA in soil through its calculated half-lives (DT_{50}) by soil layer. The kinetics model fitted the experimental data with higher determination coefficients for TBA in the upper ($r \geq 0.8662$) than in the deeper layers ($r \geq 0.6560$). The reason might be a decreased accuracy of TBA determination at trace levels. TBA half-life was shorter in the upper than in the deeper soil layers and correlated significantly negatively with the humic content. This was expected, as the humic material in surface soil generally houses diverse fungal and bacterial communities capable to degrade TBA (22), especially when the pH and temperature (25-30 °C) (28) favour their growth and activity. In this study, these optimal conditions were recorded from June to August. In soil layers poor in humus content TBA dissipation rate was higher when applied in double-the-regular amount, which points to a dominant role of movement (runoff/leaching) over biodegradation processes in that type of soil and a significantly higher risk of water pollution at enhanced herbicide application.

CONCLUSION

Regardless of the application rate, more than 80 % of the applied TBA dissipated from soil in the two months after herbicide application and 120 mm of rainfall. Three months later (at maize harvest), less than 4 % of total TBA remained in the soil, mostly in the top 20 cm rich with organic carbon that could retain the hydrophobic TBA.

Table 4 *Persistence of TBA by soil layers with respect to the applied amount of TBA*

Layer depth (cm)	DT_{50}* (days) (correlation coefficient)	
	Regular rate	Double-the-regular rate
0-10	37 (0.866)	33 (0.894)
10-20	35 (0.979)	39 (0.946)
20-30	50 (0.692)	41 (0.799)
30-50	77 (0.656)	54 (0.778)

** half-lives assuming first-order kinetics*

The loss of TBA from soil coincided with the rise in its product DET, especially in the top soil layers during the periods of low rainfall and highest soil temperatures. This points to biodegradation as the main route of TBA dissipation in humic soils.

The applied amount had no significant effect on TBA dissipation in humic soils, but in the layers with less than 1 % of organic carbon it seems to be higher.

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Dinamika disipacije terbutilazina u tlu tijekom vegetacije kukuruza

Terensko istraživanje dinamike disipacije herbicida terbutilazina (TBA) i njegova razgradnog produkta deetilterbutilazina (DET) u tlu provodilo se tijekom pet mjeseci proizvodnje kukuruza. Terbutilazin je primijenjen kao zemljišni herbicid u propisanoj dozi i u dvostruko višoj dozi od propisane. Uzorci tla periodično su skupljani pri dubinama 0-10 cm, 10-20 cm, 20-30 cm i 30-50 cm. Ostaci herbicida u tlu ekstrahirani su metanolom uz primjenu mikrovalova te analizirani HPLC-UV/DAD sustavom. Bez obzira na dozu, više od 80 % TBA rasipalo se iz prvih 50 centimetara tla dva mjeseca nakon primjene herbicida (nakon 120 mm oborina). Tri mjeseca poslije (nakon žetve kukuruza) u tlu je ostalo manje od 4 % primijenjenog TBA, uglavnom u površinskim humusnim slojevima gdje se može očekivati adsorpcija TBA na organski ugljik. Gubitak TBA iz tla praćen je porastom masenih udjela DET-a, osobito u razdoblju rijetkih oborina i najviših prosječnih temperatura tla. Najviše razine DET-a određene su u humusnim slojevima tla. Postojanost TBA u tlu značajno negativno korelira sa sadržajem humusa, što upućuje na biorazgradnju kao glavni put disipacije TBA u humusnom tlu. Doza herbicida ne utječe značajnije na brzinu disipacije u humusnom sloju tla, ali u tlu s manje od 1 % organskog ugljika može se očekivati brža disipacija TBA pri dvostrukoj nego pri propisanoj dozi.

KLJUČNE RIJEČI: *doza; deetilterbutilazin; herbicid; poljski uvjeti; postojanost*