Persistence of Simazine and Terbuthylazine in a Semiarid Soil after Organic Amendment with Urban Sewage Sludge

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The persistence of two herbicides, simazine and terbuthylazine, and appearance of their principal dealkylated chloro-*s*-triazine metabolites have been studied in agricultural soil after the addition of urban sewage sludge as organic amendment. Both herbicides and metabolites were monitored during long-term laboratory incubation (140 days) and analyzed by gas chromatography with a nitrogen−phosphorus detector (GC-NPD). Residues were confirmed by gas chromatography with a mass selective detector (GC-MSD). A sonication microextraction method was used to extract the compounds. The organic amendments used were urban sewage sludge and the humic fraction of this sludge, to increase the organic matter content of the soil from 1% to 2%. For both compounds, simazine and terbuthylazine, the degradation began earlier in the amended soils. Simazine showed a higher dissipation rate than terbuthylazine, the percentage of the former at the end of the experiment being lower than 2% in all cases, while for terbuthylazine the corresponding percentage ranged from 5% to 46%. Organic amendment, mainly its humic fraction, caused a certain stabilization of terbuthylazine in the soil, but did not greatly influence the residual amount of simazine at the end of the experiment. The periodic aeration of the soil caused a greater degradation in the case of terbuthylazine. Only mono-deethylsimazine and deethylterbuthylazine were isolated from the soil during the time the experiment lasted, while the di-deethylated metabolite of simazine was not found.

KEYWORDS: Terbuthylazine; simazine; dissipation; dealkylated chloro-s-triazine metabolites; soil; organic amendment; urban sewage sludge

INTRODUCTION

Increasing population density in towns and cities and higher living standards have led to a substantial increase in the quantity and diversity of waste products, particularly sewage sludge. Among the options available for the disposal of these wastes, controlled discharge, incineration, pyrolysis, recycling, etc. have been considered (*1*). All of these have advantages and disadvantages, although the benefits to be gained from recycling organic matter are particularly important in soils of arid zones when they are used in various strategies for soil rehabilitation (*2, 3*).

In many European countries where soils have a low organic matter content, sewage sludge is used as a soil organic amendment. Specifically, in semiarid Mediterranean areas, one of the most common ways of improving the quality of soils with a low organic matter content is the addition of fresh organic materials (such as sewage sludge). These materials are rich in

labile carbon fractions, which act as a source of energy for microorganisms, increasing soil microbial populations and their activities (*2*). This kind of organic residue improves the physical characteristics of the soil by increasing soil porosity, the water holding capacity, and the percentage of stable aggregates (*4*). The employment of the organic fraction of urban residues as soil organic amendments (or bioamendments) may be a solution to improve the low organic matter content in these areas, being able to consider those materials as "young exogenous organic matter" (*5*).

In semiarid Mediterranean areas, methods for controlling pests and diseases with use of chemical pesticides are necessary and highly effective, although pesticide behavior in soils may be modified after organic amendment, depending on the organic materials used and the pesticide properties themselves (*6*). Normally, the first effect is decreased leaching and degradation as a consequence of increased sorption (*7−13*). While this effect may limit pesticide-derived pollution, it may also impair pesticide efficacy, especially in the case of compounds applied directly to the soil, such as root-absorbed pesticides (*14*). Moreover, some organic materials used as amendment may produce soluble organic matter, which can promote pesticide

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Table 1. Characteristics of the Organic Amendment (SS), Unamended (US) and Amended Soil with Sewage Sludge (SSS) and Humic Substances (SHS) Used in This Study ($n=3$)

parameters	SS	US	SSS	SHS
	nutritional content ($\bar{x} \pm$ RSD)[a]			
total N (g kg^{-1})	30 ± 5.2	2 ± 0.5	3.2 ± 0.5	2.2 ± 0.06
N (NO$_3^-$) (mg kg^{-1})	546 ± 12.0	48 ± 3.0	102 ± 12.0	45 ± 4.0
N (NH$_4^+$) (mg kg^{-1})	2,034 ± 14.0	76 ± 14.3	165 ± 14.1	82 ± 11.2
P$_2$O$_5$ (%)	3.3 ± 0.5	0.3 ± 0.05	0.45 ± 0.05	0.3 ± 0.04
K$_2$O (%)	0.4 ± 0.08	1.2 ± 0.08	1.2 ± 0.08	1.1 ± 0.08
CaO (%)	13.8 ± 1.0	24.9 ± 1.0	23.5 ± 1.0	28.2 ± 1.7
MgO (%)	1.7 ± 0.1	3.4 ± 0.1	3.2 ± 0.1	3.1 ± 0.8
Fe (mg kg^{-1})	10245 ± 4.0	15815 ± 4.2	14820 ± 4.4	15422 ± 6.0
	organic fraction content ($\bar{x} \pm$ RSD)			
total organic carbon (%)	22.5 ± 5.0	0.9 ± 0.05	1.8 ± 0.1	1.07 ± 0.1
Na$_4$P$_2$O$_7$-extractable carbon (%)	6.2 ± 0.8	0.24 ± 0.07	0.48 ± 0.06	0.29 ± 0.02
water-soluble carbon (mg kg^{-1})	3800 ± 7.5	50 ± 8.1	60 ± 8.0	70 ± 5.0
soluble carbohydrates (mg kg^{-1})	2000 ± 6.0	10 ± 0.6	20 ± 3.0	14 ± 3.0
	heavy metals ($\bar{x} \pm$ RSD)			
Cd (mg kg^{-1})	0.7 ± 0.08	0.37 ± 0.08	0.38 ± 8.0	N.D.[b]
Hg (mg kg^{-1})	0.9 ± 0.03	0.4 ± 0.03	0.45 ± 3.0	N.D.
Cu (mg kg^{-1})	<20	<20	<20	N.D.
Ni (mg kg^{-1})	33 ± 4.0	<20	<20	N.D.
Pb (mg kg^{-1})	112 ± 7.2	<20	<20	N.D.
Cr (mg kg^{-1})	72 ± 6.1	26 ± 6.3	32 ± 6.0	N.D.
Zn (mg kg^{-1})	612 ± 3.0	52 ± 3.0	75 ± 3.1	N.D.

[a] $\bar{x} \pm$ RSD = mean ± % relative standard deviation. [b] N.D. = not determined.

desorption and enhance its apparent water solubility through stable interactions in solution between pesticide and soluble organic matter (*15*−*18*). Increased pesticide degradation may also occur by increased microbial activity in the soil after organic amendment by co-metabolism (*19*). Some studies indicate that samples from aquifers and from the deeper soil horizons (unsaturated zone) often have a certain ability to degrade pesticides, although this degradation occurs at lower rates than on the surface, where the organic matter content is higher (*20*). On the other hand, the aeration of the soil is a method used for soil bioremediation (land farming); oxygen can stimulate the microorganisms, which are able to degrade organic compounds such as pesticides (*21*).

Simazine and terbuthylazine are chloro-*s*-triazine compounds widely used in agriculture as selective herbicides. Pesticide behavior in soil depends on the soil composition and characteristics. In common with other chloro-*s*-triazine, simazine (6-chloro-N^2,N^4-diethyl-1,3,5-triazine-2,4-diamine) is converted into its hydroxy analogue in soils and undergoes dealkylation to mono-deethylsimazine (*22*, *23*). Terbuthylazine (N^2-*tert*-butyl-6-chloro-N^4-ethyl-1,3,5-triazine-2,4-diamine) is a herbicide that is mainly absorbed by plant roots. Microbial degradation proceeds mainly by de-ethylation and hydroxylation, with eventual ring cleavage (*22*−*24*).

Bearing the above in mind, we carried out an experiment to ascertain the behavior of two herbicides, simazine and terbuthylazine, when semisolid sewage sludge and the humic substances extracted from the sludge are added as organic amendment to soil with a low organic matter content. Aeration is also included as a factor in this experiment.

MATERIAL AND METHODS

Herbicides and Metabolites. Simazine, terbuthylazine, and their dealkylated chloro-*s*-triazine metabolites (mono-deethylsimazine, di-deethylsimazine, and deethylterbuthylazine) were obtained from Dr. Ehrenstorfer (Augsburg, Germany). All compounds were higher than 99.6% pure.

Soil and Organic Amendment. A Xeric Torriorthent located in Campo de Cartagena (Murcia, SE Spain), developed on a lithological

substrate of loams and characterized by its low organic matter and nutrient content, was used (**Table 1**). It had a pH (0.1 M KCl) of 8.2, and the electric conductivity was 0.25 mS cm^{-1} with 53.6% clay, 35.1% loam, 11.3% sand and a water holding capacity of 36%. An urban sewage sludge and its humic substances extracted with 0.1 M Na$_4$P$_2$O$_7$ at pH 9.8 were added to the soil. **Table 1** shows the characteristics of the sewage sludge. The organic amendment had a pH (0.1 M KCl) of 7.1 and an electrical conductivity of 2.67 mS cm^{-1}. *N* was determined by the Kjeldhal method. P, K, Ca, Mg, and heavy metals were measured after nitric−perchloric digestion by atomic absorption. The following parameters were determined by using an aqueous extract of control soil and amended soils (1:5 solid−liquid ratio): water soluble C (WSC) by oxidation with K$_2$Cr$_2$O$_7$ and measurement of absorbance at 590 nm (*25*); soluble carbohydrates by the method of Brink et al. (*26*).

The extractable carbon (humic substances) was extracted from the sewage sludge by mechanical shaking of the sample with 0.1 M Na$_4$P$_2$O$_7$ at pH 9.8 during 24 h at 37 °C in a solid/liquid ratio of 1/10; under these conditions, 6.2% of organic carbon was extracted. The extract was centrifuged for 20 min at 13200 × *g* and the supernatant was passed through a Millipore 0.22-μm membrane. The resulting humic substances fraction was dialyzed until Na$^+$, K$^+$, and Cl$^-$ were removed and finally freeze-dried.

According to Garcia et al. (*27*), taking into account the short transformation process undergone by the sewage sludge, one can suppose that the humic substances (Na$_4$P$_2$O$_7$-extractable carbon) of this material will not be identical with that of the soil and will be mainly composed of low-humified materials (like-humic substances). However, this extractable carbon is defined as a humic substance because it operates in the same way as the soil humic substances.

Elemental analysis of the HS was carried out by gas chromatography in a Hewlett-Packard 185 microanalyzer (C 47.61%; N 7.51%; H 8.13%; O 36.75%). Functional groups were determined according to the method of Schnitzer and Khan (*28*). The values obtained were as follows: total acidity, 696 mequiv/100 g; −COOH, 282 mequiv/100 g; −OH, 414 mequiv/100 g; and −C=O, 203 mequiv/100 g.

Incubation Experiments. Five hundred grams of sieved soil (<2 mm) was placed in appropriate containers. The air-dried sewage sludge was added to the soil in one third of the containers, at rate of 3% (15 g in each container), and the mixture was homogenized. In another third of the containers, humic substances were added to the soil at a rate of 200 mg of C per 100 g of soil. Mixtures in all containers were individually prepared. The rest of the containers were considered as

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"control soil". Containers with soil were spiked with 5 mL of acetone/water (20/80 v/v) solutions of simazine (499.1 mg L^{-1}) and terbuthylazine (526.8 mg L^{-1}) to obtain initial concentrations of 4.99 and 5.26 mg kg^{-1}, respectively. Triplicate treatments were incubated in a growth chamber in darkness for 140 days at 24 °C with and without manual aeration every 2 weeks. Distilled water was added to each soil mixture to bring it to 50% of its water-holding capacity. **Table 1** shows the nutritional and organic fraction contents and heavy metals of the amended soil. Throughout the incubation period water losses exceeding 10% of the initial values were compensated by the addition of fresh distilled water. After 5, 13, 34, 80, and 140 days of incubation three pots from each treatment were taken and kept at 4 °C for 2–3 days until analysis. The nomenclature used in this study was as follows: US, SSS, and SHS refer to unamended soil, soil with added sewage sludge, and soil with added humic substances, respectively, while the same initials with the suffix A refer to the same soils which were periodically aerated.

Chromatographic Conditions. *GC-NPD System.* A Hewlett-Packard 6890 equipped with a nitrogen-phosporus detector, a 6890 autosampler (Hewlett-Packard), and a split-splitless injector connected to an HP ChemStation (Hewlett-Packard) was used. The capillary column was an HP-5 (30 m × 0.32 mm i.d.) with 5% diphenyl–95% dimethyl siloxane (film thickness 0.25 μm) (Hewlett-Packard). The injector and detector were operated at 225 and 300 °C, respectively. The sample (2 μL) was injected in the splitless mode (0.75 min) and the oven temperature was programmed as follows: 70 °C for 1 min, raised to 200 °C (at 10 °C min^{-1}), and held for 3 min. Nitrogen was used as carrier and makeup gas at 2 and 8 mL min^{-1}, respectively. Hydrogen and air were used as detector gases at 3 and 60 mL min^{-1}, respectively.

GC-MSD System. A Hewlett-Packard 6890 gas chromatograph equipped with a 5973 mass selective detector (Hewlett-Packard) and split-splitless injector and connected to a Vectra 500 integrator (Hewlett-Packard) was used. A HP-5MS fused silica column (30 m × 0.25 mm i.d.) was employed, with 5% diphenyl–95% dimethyl siloxane liquid-phase (film thickness 0.25 μm) (Hewlett-Packard). The injector and interface were operated at 250 and 280 °C, respectively. The operation conditions were the following: acquisition mode SIM; EM voltage 1788 V; ionization foil temperature 230 °C; quadrupole temperature 150 °C; solvent delay 8 min; scan mass range 50–250; and selected ion monitoring (SIM), simazine *m/z* 173, 201, mono-deethylsimazine *m/z* 145, 158, 173, di-deethylsimazine *m/z* 68, 110, 145, terbuthylazine *m/z* 214, 229, and deethylterbuthylazine *m/z* 145, 186, 201. The carrier gas was He at 1.2 mL min^{-1}. The sample (2 μL) was injected in the splitless mode (60 s) and the oven temperature was programmed as follows: 90 °C for 1 min, raised to 210 °C (10 deg min^{-1}), to 240 °C (5 deg min^{-1}), and to 270 °C (30 deg min^{-1}), and held for 3 min.

Extraction Procedure and Recovery Studies. A sonication microextraction method was used. Soil samples (5 g) were extracted with 9.5 mL of Milli-Q water and 20 mL of acetonitrile. After sonication (15 min), 20 mL of dichloromethane was added and centrifuged for 10 min. The organic phase was concentrated and dissolved in 10 mL of acetone. Finally, the extract was injected in a GC-NPD system, and the identity of all compounds was confirmed by GC-MSD according to the method proposed by Navarro et al. (*29*) under the conditions specified previously. All the solvents were of suitable quality for pesticide residue analysis.

A preliminary recovery study was conducted. Soil samples without pesticide residues were homogenized and spiked with simazine and terbuthylazine at two levels (5 and 0.5 mg kg^{-1}) for both and with their metabolites at 0.5 and 0.1 mg kg^{-1}. After evaporation of the spiked solvent, the samples were allowed to equilibrate for 120 min and then processed according to the above procedure. Five replications were used in each case.

RESULTS AND DISCUSSION

Analytical Determination of Herbicide Residues. The values obtained by linear regression for the five studied compounds show a high degree of correlation between concentration and area ($r > 0.995$). The detection limits, LOD (signal-to-noise, ratio = 3), were 1 pg for di-deethylsimazine, 5 pg for simazine, terbuthylazine, and deethylterbuthylazine, and 10 pg for mono-deethylsimazine. In all cases, recoveries were higher than 89% with the exception of di-deethylsimazine (74%). The coefficient of variability did not exceed 6% in the most unfavorable case. The limits of quantitation (LOQ) for the NP detector were 1 ppb for di-deethylsimazine, 5 ppb for simazine, terbuthylazine, and deethylterbuthylazine, and 10 ppb for mono-deethylsimazine. In the case of the MS detector (in SIM mode), the limits of quantification were similar. Cleanup was not necessary. It appears that for samples with an organic matter content <5% it is possible to process uncleaned extracts as stated by Molins et al. (*30*).

Characterization of Organic Amendment and Soil. The nutritional content of the sludge (**Table 1**) shows its suitability for use as an amendment, bearing in mind the N and P contents. This high N content is normal because urban depuration sludge is predominantly of proteic origin. The P content comes fundamentally from the polyphosphates contained in detergents, which, although hypothetically biodegradable, can still be found in sludge. On the other hand, many of the macronutrients in the sludge used are in organic form, which would explain its low inorganic N (NO_3^- and NH_4^+) content compared with total N. These products, therefore, should not be considered as fertilizers in the strict sense of the term, although they may be useful as fertilizers, particularly because they can contribute the macronutrients to the soil in a gradual way. This result is interesting from an agronomic point of view.

The organic fractions clearly showed that our material had a high percentage of total organic carbon (**Table 1**), as well as of humic substance carbon (extractable with sodium diphosphate). As a consequence, the sludge could be considered as suitable for the organic amendment of soils. At the same time, it had a large amount of labile organic matter (water-soluble carbon and soluble carbohydrates), which is to be expected if we bear in mind that it is a fresh material, which has not stabilized.

Finally, the presence of heavy metals in the sludge was not problematic (**Table 1**). The values were within the limits established by the Spanish legislation for using this material in soil (*31*).

The soil used in this study was a clay loam soil. Among the general characteristics (**Table 1**) is its low organic carbon content (total OC: 0.9%), along with relatively high N content (total N: 2 g kg^{-1}), which seems to be related to agricultural uses in this area. The substantial presence of carbonates probably explains the alkaline pH (8.6). The electrical conductivity value (0.25 mS cm^{-1}) shows that there is no risk of salinization.

Once the soil was amended with the urban sewage sludge, there was a 60% increase in the total N content, while the total organic Carbon, extractable carbon, and soluble carbohydrate contents doubled (1.8%, 0.48%, and 20 mg kg^{-1}, respectively) and water-soluble carbon increased slightly. The other parameters showed little variation, as can be seen in **Table 1**. The increase in the heavy metal content compared with the unamended soil was not significant.
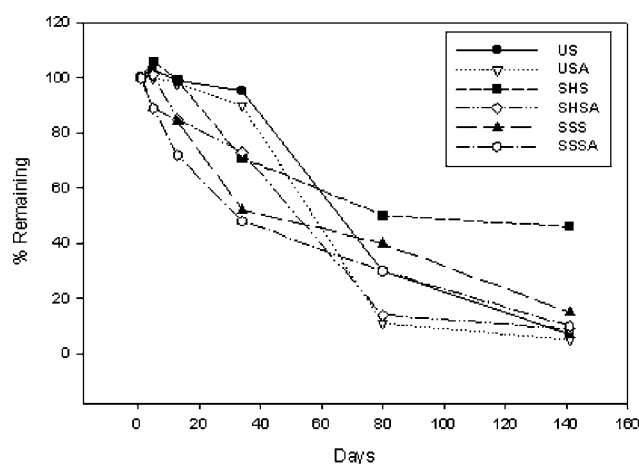
In the case of the soil amended with humic substances, the nutritional content remained constant, while little increments in the organic fraction contents were observed, although the value of soluble carbohydrates was higher (14 vs 10 mg kg^{-1}).

Effect of Organic Amendment on Herbicide Kinetics. A greater dissipation rate was observed for simazine than for terbuthylazine, as can be seen in **Figures 1** and **2**, where the time-course of herbicide residues is shown. The percentages remaining at the end of the experiment (140 days) were lower than 2% in the case of simazine while the final concentration

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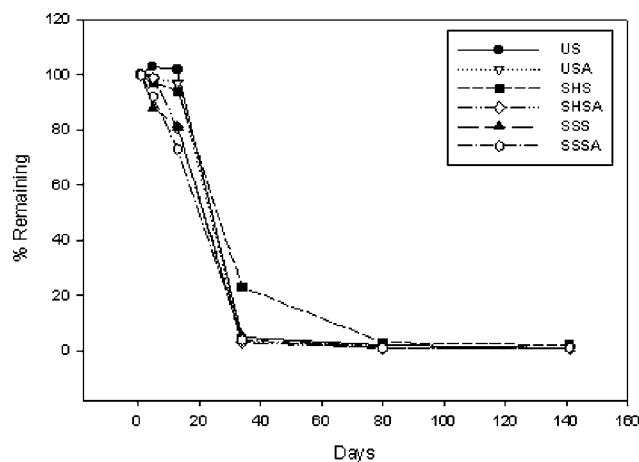
Figure 1. Percentage of simazine remaining in unamended (US) and amended soil with sewage sludge (SSS) and humic substances (SHS) with time. The suffix A refers to aerated soils.

Figure 2. Percentage of terbuthylazine remaining in unamended (US) and amended soil with sewage sludge (SSS) and humic substances (SHS) with time. The suffix A refers to aerated soils.

varied from 5% (USA) to 46% (SHS) of the initial amount in the case of terbuthylazine.

Tables 2 and **3** show the residual values found during the incubation study for simazine and terbuthylazine, respectively. The dissipation of both herbicides could be described by pseudo

first-order kinetics: $\ln R_t = \ln R_0 - kt$, where R_0 and k denote y-intercept values and the slope of the dissipation lines, respectively, R_t is the concentration of pesticide in soil, and t is the postapplication time in days (*32*). The kinetic parameters obtained can be seen in **Table 4**.

The initial concentration of simazine (day 0, 1 h) ranged from 4.77 to 5.02 mg kg^{-1}. In all cases dissipation was rapid, lowering residual values to ≤0.25 mg kg^{-1} in the first 34 days, with an exception in SHS, where 1.17 mg kg^{-1} was found at this time. After 80 days residual levels were similar in all conditions and showed values of 0.04 to 0.09 mg kg^{-1} at the end of the experiment (140 days). The disappearance of this compound followed a first-order kinetics of two phases, consisting of an initially fast dissipation stage (0−34 days) followed by a much slower persistence phase (34−140 days) as corroborated by the calculated rate constants. The half-lives varied from 7 days (SSS and SSSA) to 14 days (SHSA) in the first phase ($r = 0.93-0.96$) and from 28 to 110 days for SHS and USA, respectively, for the second phase ($r = 0.76-0.91$). For the overall period ($r = 0.84-0.97$) the range was 18−22 days. These findings denote that the organic amendment used in this experiment had no marked influence on the behavior of simazine in the soil at the end of the incubation period, although its degradation begins earlier in the soil amended with sewage sludge compared with the untreated soil. Thus, at day 13, all simazine applied (97−103%) remained in the untreated soil, whereas only 80% and 73% of the herbicide applied was extracted from the SSS and SSSA treatments, respectively.

For terbuthylazine, the initial concentration (day 0, 1 h) ranged from 4.87 to 5.01 mg kg^{-1}. In contrast with simazine, the dissipation of terbuthylazine was relatively slow. According to the calculated rate constant values there was no clear difference between the dissipation and persistence phases. In the first period ($r = 0.89-0.99$), terbuthylazine reached higher levels in unamended soils (US, $t_{1/2} = 315$ days and USA, $t_{1/2} = 217$ days) than in the amended soils (with or without aeration) in which $t_{1/2}$ ranged from 32 to 69 days. In the second phase ($r = 0.91-1.00$), the maximum half-life (178 days) was for the soil amended with humic substances (SHS), while in the other soils the values ranged from 28 to 57 days. Although disappearance of terbuthylazine in amended soil begins earlier (at the end of the first phase) than in unamended soil, after 140 days, the residual amounts of this compound in amended soil were slightly greater than those found in unamended soil. This behavior may

Table 2. Concentration of Simazine and Mono-deethylsimazine (Mean ± % Relative Standard Deviation, $n = 3$) in Soil during the Incubation Process under Different Conditions

soil[a]	days of incubation =					
	0	5	13	34	80	140
	simazine (mg kg^{-1})					
US	4.77 ± 3.3	4.92 ± 3.9	4.90 ± 2.0	0.25 ± 8.0	0.10 ± 10.0	0.09 ± 22.2
USA	4.90 ± 4.1	4.87 ± 15.2	4.75 ± 6.5	0.16 ± 25.0	0.09 ± 11.1	0.08 ± 17.5
SHS	4.98 ± 3.4	4.81 ± 6.6	4.70 ± 5.5	1.17 ± 20.5	0.17 ± 22.9	0.08 ± 25.0
SHSA	4.90 ± 3.9	4.86 ± 3.1	3.97 ± 3.5	0.16 ± 18.2	0.04 ± 18.0	0.04 ± 20.0
SSS	5.02 ± 3.2	4.44 ± 2.7	4.06 ± 6.6	0.23 ± 21.7	0.05 ± 20.0	0.06 ± 22.0
SSSA	4.89 ± 3.3	4.49 ± 8.5	3.58 ± 4.2	0.18 ± 11.1	0.06 ± 16.7	0.05 ± 20.0
	mono-deethylsimazine (mg kg^{-1})					
US	BDL[b]	BDL	BDL	0.35 ± 31.8	0.24 ± 19.4	0.12 ± 25.0
USA	BDL	BDL	0.25 ± 10.2	0.26 ± 15.4	0.24 ± 20.5	0.28 ± 29.3
SHS	BDL	BDL	BDL	0.25 ± 12.1	0.23 ± 8.7	0.07 ± 7.8
SHSA	BDL	BDL	BDL	0.20 ± 7.5	0.29 ± 5.2	0.29 ± 20.5
SSS	BDL	0.17 ± 3.1	0.35 ± 4.3	0.68 ± 24.0	0.42 ± 14.6	0.36 ± 19.1
SSSA	BDL	0.26 ± 15.4	0.73 ± 5.7	0.91 ± 18.4	0.58 ± 9.1	0.48 ± 17.8

[a] US = unamended soil. USA = unamended soil + aeration. SHS = soil + humic substance. SHSA = soil + humic substance + aeration. SSS = soil + sewage sludge. SSSA = soil + sewage sludge + aeration. [b] BDL = below detection limit.

Table 3. Concentration of Terbuthylazine and Deethylterbuthylazine (Mean ± % Relative Standard Deviation, $n = 3$) in Soil during the Incubation Process under Different Conditions

| soil[a] | days of incubation = | | | | | |
	0	5	13	34	80	140
	terbuthylazine (mg kg⁻¹)					
US	4.92 ± 1.8	5.06 ± 2.0	4.89 ± 5.5	4.65 ± 2.8	1.48 ± 21.0	0.34 ± 11.8
USA	4.94 ± 2.2	4.96 ± 6.3	4.86 ± 2.7	4.45 ± 2.7	0.55 ± 10.9	0.24 ± 16.7
SHS	4.87 ± 5.5	5.18 ± 4.6	4.83 ± 5.0	3.46 ± 10.1	2.45 ± 17.9	2.26 ± 15.9
SHSA	5.01 ± 4.4	5.06 ± 3.3	4.24 ± 4.5	3.66 ± 10.9	0.69 ± 19.0	0.47 ± 20.7
SSS	4.87 ± 3.7	4.32 ± 3.0	4.11 ± 8.5	2.55 ± 12.9	1.95 ± 18.2	0.72 ± 4.2
SSSA	4.92 ± 2.4	4.40 ± 3.2	3.54 ± 1.1	2.35 ± 7.6	1.46 ± 12.2	0.48 ± 17.1
	deethylterbuthylazine (mg kg⁻¹)					
US	BDL[b]	BDL	BDL	0.42 ± 8.3	0.49 ± 9.5	0.48 ± 4.4
USA	BDL	BDL	BDL	0.42 ± 3.6	0.50 ± 4.0	0.52 ± 21.4
SHS	BDL	BDL	0.25 ± 13.6	0.30 ± 6.6	0.44 ± 8.5	1.07 ± 25.9
SHSA	BDL	BDL	0.52 ± 3.1	0.78 ± 9.2	0.59 ± 28.2	0.68 ± 17.5
SSS	BDL	0.62 ± 17.3	0.79 ± 11.9	1.00 ± 19.0	1.57 ± 15.4	1.78 ± 9.1
SSSA	BDL	0.50 ± 14.7	1.25 ± 14.0	1.24 ± 14.0	1.35 ± 24.5	1.57 ± 20.7

[a] US = unamended soil. USA = unamended soil + aeration. SHS = soil + humic substance. SHSA = soil + humic substance + aeration. SSS = soil + sewage sludge. SSSA = soil + sewage sludge + aeration. [b] BDL = below detection limit.

**Table 4.** Parameters Obtained from the First-Order Kinetics for Simazine (Sz) and Terbuthylazine (Tz)

| herbicide | parameter[a] | phase[c] | soil[b] | | | | | |
			US	USA	SHS	SHSA	SSS	SSSA
Sz	r	first	0.932	0.937	0.944	0.952	0.953	0.960
		second	0.872	0.906	0.948	0.825	0.759	0.892
		overall	0.868	0.842	0.969	0.877	0.876	0.882
	SE of Est.	first	0.655	0.731	0.289	0.629	0.554	0.549
		second	0.389	0.220	0.620	0.641	0.764	0.443
		overall	1.112	1.268	0.512	1.285	1.193	1.167
	R_0	first	7.64	8.24	6.05	7.88	7.29	7.24
		second	0.29	0.18	2.01	0.18	0.24	0.22
		overall	3.37	3.11	4.80	3.06	3.13	2.96
	rate constant (days⁻¹)[d]	first	$92.1 \times 10^{-3\,d}$	$63.4 \times 10^{-3\,d}$	$56.4 \times 10^{-3\,d}$	$48.2 \times 10^{-3\,e}$	$94.5 \times 10^{-3\,e}$	$101.6 \times 10^{-3\,e}$
		second	9.2×10^{-3}	6.3×10^{-3}	24.6×10^{-3}	12.5×10^{-3}	11.9×10^{-3}	11.6×10^{-3}
		overall	$31.8 \times 10^{-3\,e}$	$32.4 \times 10^{-3\,e}$	$32.5 \times 10^{-3\,f}$	$38.4 \times 10^{-3\,e}$	$35.4 \times 10^{-3\,e}$	$35.6 \times 10^{-3\,e}$
	$t_{1/2}$ (days)	first	8	11	12	14	7	7
		second	75	110	28	55	58	60
		overall	22	21	21	18	20	19
Tz	r	first	0.891	0.957	0.936	0.964	0.988	0.997
		second	1.000	0.949	0.911	0.912	0.971	0.989
		overall	0.979	0.969	0.941	0.964	0.988	0.994
	SE of Est.	first	0.021	0.018	0.078	0.051	0.055	0.029
		second	0.009	0.665	0.133	0.631	0.225	0.167
		overall	0.247	0.373	0.138	0.318	0.122	0.102
	R_0	first	5.00	4.99	5.23	5.06	4.90	4.84
		second	10.58	8.12	3.71	5.11	4.25	4.22
		overall	6.21	6.01	4.83	5.27	4.67	4.59
	rate constant (days⁻¹)[c]	first	2.2×10^{-3}	$3,2 \times 10^{-3\,e}$	$11.3 \times 10^{-3\,d}$	$10.1 \times 10^{-3\,e}$	$18.8 \times 10^{-3\,e}$	$21.8 \times 10^{-3\,f}$
		second	$24.5 \times 10^{-3\,f}$	26.8×10^{-3}	3.9×10^{-3}	18.6×10^{-3}	12.1×10^{-3}	15.1×10^{-3}
		overall	$19.5 \times 10^{-3\,f}$	$23.9 \times 10^{-3\,f}$	$6.3 \times 10^{-3\,f}$	$18.9 \times 10^{-3\,f}$	$13.0 \times 10^{-3\,f}$	$15.9 \times 10^{-3\,f}$
	$t_{1/2}$ (days)	first	315	217	61	69	37	32
		second	28	26	178	37	57	46
		overall	35	29	110	37	53	44

[a] r = regression coefficient. SE of Est. = standard error of estimation. R_0 = theoretical initial residue. Rate constant and $t_{1/2}$ in half-lives. [b] US = unamended soil. USA = unamended soil + aeration. SHS = soil + humic substance. SHSA = soil + humic substance + aeration. SSS = soil + sewage sludge. SSSA = soil + sewage sludge + aeration. [c] First phase: 0–34 days. Second phase: 34–140 days. Overall: 0–140 days. [d] $P < 0.1$. [e] $P < 0.05$. [f] $P < 0.01$.

be due to the highest microbial activity taking place during the first phase, in contrast with the second phase, where the herbicide sorption was probably increased and microbial activity decreased. For the overall period ($r = 0.94 - 0.99$) the range was from 29 to 110 days, corresponding to the highest value to SHS. The different behavior of terbuthylazine in the amended soil (SHS vs SSS) could be explained by the adsorption capacity of the humic substances, which is related to their composition and structural and functional properties (33, 34).

The above results are in agreement with those of other published papers. Thus, the addition of compost increases the soil organic matter content (14), which would partly explain the increased stabilization of some s-triazine herbicide residues. This is especially true for the most highly sorbed herbicides and could indicate that stabilization occurs mainly through sorption processes. For such compounds, in general the remaining percentage present as extractable residues decreases with the proportion of mineralized percentage while the proportion of bound residues increases. This indicates that bound residue formation is related to biological activity and to the biotransformation of the herbicides, and is indicative of the capacity of the microflora to use those herbicides or their metabolites as

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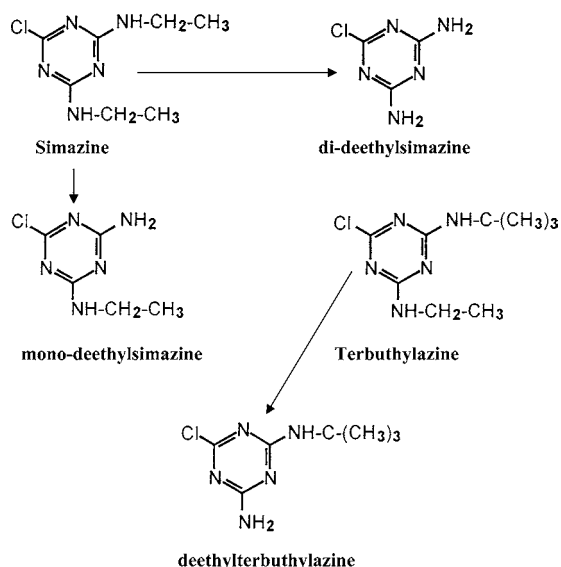
Figure 3. Partial metabolic pathways (dealkylated chloro-*s*-triazine compounds) for simazine and terbuthylazine in soils.

metabolic substrates. Working with *Pseudomonas* sp strain ADP inoculated into soils, Jacobsen et al. (*35*) also described a sharp increase in the mineralization of terbuthylazine, which was only limited by the desorption process. A study carried out by Fernández et al. (*34*) showed that the addition of compost increases microbial biomass and herbicide sorption. Similar results were obtained by Graber et al. (*36*) concerning the behavior of four *s*-triazine herbicides including simazine and terbuthylazine after sludge amendment. Also, Cox et al. (*37*) have demonstrated that soils amended with a highly humified amendment sorbed simazine to a greater extent than the untreated soils.

Metabolic Pathways of Simazine and Terbuthylazine. There is extensive published information concerning the metabolism of simazine and terbuthylazine in plants and animals (*23*), but less on their degradation in soil. In common with other chloro-*s*-triazines, simazine and terbuthylazine are transformed in soil and undergo dealkylation to form deethylated products, as can be seen in **Figure 3**.

For simazine only the mono-dealkylated product was detected (**Table 2**). Mono-deethylsimazine appeared in the sludge-amended soil 5 days after application of the active ingredient. The maximum amount recorded for this metabolite was 0.68 and 0.91 mg kg^{-1} for the nonaerated (SSS) and aerated soils (SSSA), respectively, 34 days after application. At the end of the experiment the concentrations ranged from 0.36 to 0.48 mg kg^{-1} for SSS and SSSA, respectively. In the case of the soil amended with humic substance (SHS and SHSA), the metabolite appeared 34 days after treatment, with its highest concentration reaching 0.29 mg kg^{-1} (140 days) in the aerated soil (SHSA). This compound was also found in the unamended soil. The final concentrations after 140 days of incubation were 0.12 and 0.28 mg kg^{-1} for the nonaerated (US) and aerated (USA) soils, respectively. In all cases the amounts of metabolite detected at the end of the experiment were <0.5 mg kg^{-1}, which corresponds to about 10% of the parent compound and agrees with other studies. Barriuso et al. (*14*), working with simazine ^{14}C-labeled on the aromatic ring, found that the total mineralized, total extractable, and total nonextractable radioactivity percentages of initial ^{14}C were 63.6, 1.4, and 20.3, respectively, showing that this molecule undergoes rapid mineralization under conditions of the experiment.

In the case of terbuthylazine, deethylterbuthylazine was found as a metabolite in all treatments (**Table 3**). At the end of the incubation period (140 days) deethylterbuthylazine concentration was highest in the sludge-amended soil not subjected to aeration, SSS (1.78 mg kg^{-1}), although the value found for the aerated (SSSA) soil (1.57 mg kg^{-1}), was similar. For the unamended soil (US) and soil amended with humic substance (SHS), the concentrations at the end of the experiment varied from 0.48 to 1.07 mg kg^{-1}, respectively. In the SSS and SSSA treatments, the metabolite appeared 5 days after application of the herbicide. The percentage of metabolite detected after 140 days ranged from 10% to 37% of the parent compound, higher percentages of metabolite than in the case of simazine. The behavior of terbuthylazine could be related to its slow mineralization. Thus, in a study carried out by Dousset et al. (*24*) with ^{14}C-terbuthylazine uniformly labeled with ^{14}C on the *s*-triazine ring in three soils, degradation was slow and led to a very limited mineralization (0.9% to 1.2% of the applied radioactivity), while a large proportion of the compound became nonextractable (33.5% to 43.1%). The decrease in terbuthylazine concentration in that study was accompanied by an increase in deethylterbuthylazine concentration.

Experiments with terbuthylazine, atrazine, and simazine with fungus (*38*) and bacteria (*39*) showed that removal of the ethyl side chain is the preferential reaction and that this might depend on the mass of the second alkyl group. Many studies have indicated that the major transformation products for *s*-triazines are mono-dealkylated compounds (*20, 24, 40−42*) which agrees with our findings.

CONCLUSIONS

From this study involving unamended soils and soils amended with urban sewage sludge in laboratory conditions after treatment with simazine and terbuthylazine, it can be concluded that the organic fractions of the sludge have high total organic and humic substance carbon contents. It may therefore be considered as a suitable organic amendment for soils. A greater dissipation rate was observed for simazine than for terbuthylazine. Periodic aeration of the soil led to a more pronounced degradation in the case of terbuthylazine, presumably because of the increased activity of soil microorganisms, while no significant differences were observed for simazine. Organic amendment increased the persistence of terbuthylazine in the soil due possibly to the strong adsorption capacity of this herbicide, but did not influence the behavior of simazine at the end of the experiment to any great extent. Only mono-dealkylated compounds (mono-deethyl-simazine and deethylterbuthylazine) were detected as metabolites.

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