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Fate and degradation of triasulfuron in soil and water under laboratory conditions

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The behavior and fate of triasulfuron (TRS) in water and soil systems were examined in laboratory studies. The degradation of TRS in both buffer solution and soil was highly pH-sensitive. The rate of degradation could be described with a pseudo first-order kinetic and was much faster at pH 4 than at pH 7 and 9. Aqueous hydrolysis occurred by cleavage of the sulfonylurea bridge to form 2-(2-chloroethoxy) benzenesulfonamide (CBSA) and [(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino] (AMMT). AMMT was unstable in aqueous solutions in any pH condition but it degraded more quickly at pH 4 and 9. CBSA did not degrade in aqueous solutions or in enriched cultures but it underwent a quick degradation in the soil. The rates of TRS degradation in sterile and non-sterile soils were similar, suggesting that microorganisms played a minimal role in the breakdown process. This hypothesis is supported by the results of studies on the degradation of TRS by enriched cultures during which the molecule underwent a prevalently chemical degradation.

Keywords: Triasulfuron; unsterile soil; sterile soil; water; degradation; degradation products; CBSA; AMMT.

Introduction

Triasulfuron [3–6 methoxy-4-methyl-1,3,5-triazin-2-yl)-1-(2-(2-chloroethoxy)phenylsulfonyl)-urea] (TRS) is a sulfonylurea herbicide active against broad leaf weeds in wheat and barley.[1] Its use is increasing due to its low application rate (5–10 g/ha). Sulfonylurea herbicides most commonly dissipate through chemical hydrolysis and microbial degradation while volatilization is not significant. Pusino et al.[2] provide basic information about the photoreactivity of TRS showing a faster degradation under irradiation at 254 nm (half-life = 2.0 min) than at 366 nm (half-life = 9.7 days). They concluded that photolysis could contribute to the detoxification of TRS in water.

It has been found that the degradation rate of TRS in soil decreases with increasing pH and hydrolysis is the dominant pathway in acidic soil. However, some authors have predicted that significant quantities of TRS residues will be present 12–36 months after application.[3,4] This might be due to the fact that soil pH and organic matter content have a great influence on the adsorption and degradation of TRS. Previous studies with TRS indicated half-lives ranging from 11–91 days in soil with different pH, organic matter content and in various moisture and temperature conditions. Oppong and Sagar[5] observed a half-life of 11 days at 30°C in an acidic soil (pH 5.2) and Singh et al.[6] reported half-lives of 18, 22 and 25 days in soils with pH 6.6, 8.2 and 8.3 respectively. Dinelli et al.,[7] found a half-life of 91 days in a soil (pH 8) treated with a commercial formulation of TRS (70% WG). Numerous studies indicated the involvement of soil microorganisms in the breakdown of TRS, comparing half-lives in autoclaved and microbiologically active soil.[5,6,8] However, it has been demonstrated that autoclaving alters some soil properties e.g the specific surface[9], as well as the amount of soluble salts or organic matter[10,11] thus modifying the catalytic capacity of the soil. Several authors based their pesticide degradation studies on the sterilization of soil with epoxides, such as ethyleneoxide, because these substances induce the least chemical alterations.[11–13]

Although numerous studies have been carried out to evaluate the degradation of TRS in sterile and non-sterile soils, to our knowledge none used a sterilization method that not alters the soil chemical-physical characteristics. Moreover, limited information is available on the behavior of TRS by-products in soil and water.

The main objective of the present work was to investigate the role of microflora in the degradation of TRS, comparing both its degradation rate in non-sterile soil and in soil

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sterilized with ethylene-oxide as well as evaluating the capability of selected microbial consortiums to use TRS as their only source of energy and carbon. The degradation rate of TRS was also determined in a sterile buffer solution at different pH so as to better understand the role of biotic and/or abiotic degradation in its fate. In addition, the by-products of TRS were isolated and identified and experiments were performed on their degradation in water at different pH, in soil, and by enrichment cultures.

Materials and methods

Chemicals and soils

Triasulfuron (TRS, 99% purity) and pesticide-grade solvents were supplied by Sigma Aldrich (Milan, Italy).

The soils used in this study were obtained from agricultural plots situated in Carmagnola, Savigliano and Fossano (Piedmont, Italy) which had never been treated with sulfonylurea herbicides. The soils were sampled to a depth of 0–25 cm, partially air dried, passed through a 2 mm sieve and stored for less than one week at + 4°C to maintain any biological activity. The main characteristics of the three soils are shown in Table 1.

'BARD' Steril Peel (7.6 cm) Packaging system polythene bags were used to contain the soil samples for sterilization. Eight hour applications of 10% ethylene oxide in CO_2atmosphere at 4. 10^5 Pa pressure, 65% relative humidity (RH), 40°C temperature and 3 days desorption time were run. This procedure ensures a good biocidal effect[12]. Sterile conditions were maintained performing all operations in a laminar vent hood and using sterile materials.

Stability of triasulfuron in water

Glass flasks containing10 mL buffer solution with respective pH of 4 (citric acid/sodium hydroxide solution/sodium chloride), 6 (citric acid/sodium hydroxide solution), 7 (potassium dihydrogen phosphate/disodium hydrogen phosphate), and 9 (sodium teraborate/hydrochloric acid), and 2 mL of TRS aqueous solution (50 ppm) were prepared. The tests were carried out in sterile conditions placing the flasks containing the buffer solution in an autoclave at 120°C for 15 min. The TRS solution was sterilized by passing through a 0.45 μm Millipore filter to avoid degradation due to the heat. The samples were placed in a thermostat in the dark at 25°C. Two individual flasks of each pH were removed at 0, 7, 14, 21, 28, 56, and 118 days and analyzed for TRS residual concentration. The test was repeated twice.

Table 1. Selected chemical and physical properties of the soils.

	(%)					
Soils	*pH*	*O. C.*	*Clay*	*Silt*	*Sand*	*Water-holding capacity*
Fossano	6.1	0.91	9.9	42.9	47.2	41
Carmagnola	7.9	0.94	10.6	48.6	40.8	37
Savigliano	6.3	1.01	9.2	52.7	38.1	37

Stability of triasulfuron in soil

Degradation tests were performed in both sterile and non-sterile conditions to verify the degradability of TRS in the soil and to establish whether the presence, or absence, of bacterial flora produced different kinetics. The tests were performed on the soils coming from Carmagnola, Savigliano and Fossano (Piedmont, Italy). The determination of the degradation kinetics in sterile soil was carried out on the Carmagnola soil.

One mL of TRS aqueous solution (25 ppm) was placed with 50 g of soil (on a air dry basis) in 250 mL polyethylene bottles to obtain a TRS soil concentration equal to 0.5 mg kg^{-1} of dry soil and enough water was added to take the soil to 60% of its Water Holding Capacity (WHC). The soil moisture content in each bottle was maintained at 60% of WHC by addition of water at least weekly throughout the study. Sterile conditions were maintained by performing all operations in a laminar vent hood and using sterile materials. For the tests on non-sterile soil, the bottles were partially closed to allow for an exchange of gases, while for the sterile soil tests the bottles were hermetically closed to avoid contamination. The samples were incubated in a thermostat, in the dark, at 25°C.

For both tests, three replicates were taken immediately after addition of the herbicide and then after 3, 7, 14, 21 and 35 days. Each test was repeated twice.

Triasulfuron analysis

The extraction of TRS was performed on 25 g of soil taken from the degradation test after being carefully stirred. 7.5 g of diatomee powder (Extrelut, Merck) was added to favor contact between the soil and the extractant. The extraction was carried out by means of three consecutive washings with 75 mL acetonitrile. Each time the solvent was added the suspension was agitated for 30 min on a mechanical stirrer and centrifuged at 3000 rpm for 15 min. The three supernatant were passed through n. 4 Whatman filters, placed together in a 250 mL round bottom flask and brought to dryness in a rotary evaporator. The dry residue was resolubilized in 2 mL of acetonitrile and water (50:50 v/v) and passed through a 0.45 μm nylon filter, then analyzed by means of liquid chromatography (LC). A Perkin Elmer Model 250 Liquid Chromatograph was used with a UV/Visible detector set at 220 nm and a Supelcosil LC-8 column (25 cm × 4.6 mm, 5 μm), at room temperature. The mobile phase consisted in acetonitrile (solvent A = 33%) and water which had been acidified to pH 3 with ortophosphoric acid (solvent B = 67%); the flow was 1 mL/min.

When the TRS had been eluted, any compounds remaining in the column were removed, taking the mobile phase to 20% solvent A and 80% solvent B in 10 minutes and keeping the concentration the same for another 10 minutes. This method of analysis made it possible to recover 93.4 ± 3% TRS from the soil with a sensitivity limit of 0.002 mg kg^{-1} dry soil (fortification levels = 0.05, 0.1, 0.5 mg kg^{-1}). The aqueous solution was analyzed after a 1:5 dilution of the sample with a mixture of acetonitrile and water (50:50 v/v). The samples were then analyzed using liquid chromatography as above. In this case it was not necessary to wash the column after the elution of TRS.

Separation and identification of breakdown products of triasulfuron

While analyzing the hydrolysis test samples at pH 4 it was possible to observe the appearance of two compounds which were not present in the initial chromatograms. Given that the concentration of these two compounds increased as TRS disappeared, it seemed plausible to hypothesize that they were produced by the degradation of the herbicide. To obtain a large enough quantity of the two compounds to identify them, flasks were prepared containing an aqueous solution of TRS with a 50 ppm concentration and pH 4. The flasks were kept in the dark, in a thermostat, at a constant temperature of 25°C for one month. The two compounds were then extracted by liquid-liquid extraction according to the following procedure.

The aqueous solution, acidified to pH 2 with 1N HCl was extracted twice with dichoromethane. The organic phase, containing product 1, was brought to dryness in a rotary evaporator and then redissolved in 10 ml acetonitrile. The aqueous phase was brought to pH 11 with 1N NaOH, then extracted twice with dichloromethane. The organic phase, containing product 2, was brought to dryness in a rotary evaporator and then redissolved in 10 ml acetonitrile.

The mass spectra of triasulfuron and degradation product 1 were obtained, by LC-MS using a Finnigan LCQ system. The column was a Supelcosil LC-18, 15 cm x 4.6 mm, 5 μm. The mobile phase was methanol/20 mM ammonium acetate (60:40, V/V). The mass spectrometer was equipped with an ESI ionization source. The analysis mode was in positive ionization.

The mass spectrum of degradation product 2 was obtained by gas chromatography-mass spectrometry GC-MS using a Hewlett Packard Mod. 5989 instrument. The chromatographic separation was performed using a HPS capillary column of 30 cm × 0.25 mm with a phase thickness of 0.25 μm. The carrier gas was helium. The oven temperature was kept at 35°C for 3 min and then raised from 35°C to 85°C at a speed of 10°C/min and finally from 80°C to 250°C at a speed of 15°C/min; a constant temperature of 250°C was then maintained for 20 min. The mass detection was in electronic impact (70 eV).

Stability of breakdown products of triasulfuron in water

To evaluate the chemical stability of the two products coming from the degradation of TRS, hydrolysis tests were set up at pH 4, 7 and 9 using the same method as described above for TRS. The test was repeated twice.

The analyses were performed using a Perkin Elmer Model 250 Liquid Chromatograph with a wavelength UV/VIS detector and a Supelcosil LC-8 column (25 cm × 4.6 mm, 5 μm), at room temperature.The analytical conditions were as follows: mobile phase, water acidified to pH 3 with ortophosphoric acid (63%) and acetonitrile (37%); flow, 1 mL/min; detector wavelength, 220 nm (the retention times of product 1 and product 2 were 3.4 and 3.9 respectively). Quantitative determination of the breakdown products was performed comparing the LC peak areas in the samples with a calibration curve obtained from the pure compounds isolated from the buffer solution at pH 4 as described above.

Stability of product 1 in soil

Due to its instability product 2 was isolated at low concentrations which were insufficient to carry out any other experiment. The work was then continued with the sole product 1. Carmagnola soil was used for this test. Twenty five g of soil (on a dry basis) were placed in a 250 mL polyethylene bottle then 1 mL of aqueous solution of product 1 was added (10 ppm) to obtain a concentration equal to 0.4 mg kg^{-1} of dry soil; enough water to take the soil to 60% of its WHC was added. The soil moisture content in each bottle was maintained at 60% of WHC by addition of water at least weekly throughout the study. After partial closure of the bottles, the samples were placed in a thermostat at 25°C in the dark. Three replicates were taken immediately after addition of the product 1 and then after 3, 7, 14, 21 and 35 days. Product 1 was extracted from the soil using the same procedure as for the determination of TRS (recovery was 96.7%± 4.4). The conditions for the analyses were the same as those reported above. The test was repeated twice.

Enrichment cultures

To identify those microorganisms able to degrade TRS and product 1, enrichment cultures were set up using different inocula. These were: (a) active sludges from a paper mill purification plant (active sludges 1); (b) leacheate from a waste tip; (c) active sludges from a sewage plant (active sludges 2); (d) sediment from a rice-field which had previously been treated with another sulfonylurea (cinosulfuron); and (e) soil from an area previously treated with TRS. For each inoculum three 300 mL flasks were prepared in sterile conditions each containing 80 mL of liquid mineral medium,[14] 20 mL of a 50 ppm aqueous solution of TRS or product 1 as sole source of carbon, and 5 mL of inoculum (5 g in the case of the soil). The flasks were then placed on a mechanical

shaker. The concentration of the main ingredients was determined extracting 5 mL of the culture broth from each flask after 0, 7, 14, 21, 28, 56, and 118 days.

The selection of microorganisms which were able to degrade the molecules was enhanced by two successive transplants using 10 mL of culture broth taken from the tests in progress. After the second transplant, either glucose (1 mL of a 10% aqueous solution) or peptone (6 mL of a 20% aqueous solution) was added to each flask to improve microbial development.

For all tests, least significant differences were determined after analysis of variance (ANOVA), employing standard *t*-test to determine the mean life lives not different at the 0.05 level of significance.

Results and discussion

Stability of triasulfuron in water

The hydrolysis tests in the pH range 4–9 followed pseudo first-order kinetics (Table 2). The reaction was fast at acidic pH and much slower at neutral and basic pH with a drastic decrease of the rate constant from pH 6 to pH 7. This behavior agreed with that observed by Braschi et al.[15] The lower hydrolysis rate constants for buffer solutions at pH 7 and 9 show that the molecule is relatively stable in these pH conditions and less likely to be degraded abiotically than in more acidic conditions.

Sarmath et al.[16] developed a model to study the functional relationship between pH and the hydrolysis rate of sulfonylurea herbicides based on the propensity of nonionic and anionic species of the herbicides to undergo hydrolysis. They showed that hydrolysis in water is 100 times faster in acidic conditions (i.e. when the molecule is undissociated) than in neutral or alkaline conditions when it is in anionic form.

The estimated half-lives were found to be different from those reported in other studies. For example, in this study, the half-lives in aqueous buffer solution were found to be about 4, 89, 289 and 365 days at pH 4, 6, 7 and 9 respectively and at a temperature of 25°C. In contrast, Braschi et al.[15] reported the hydrolysis half-life times to be about 8, 27, 492 and 387 days with the same pH and temperature conditions. Dinelli et al.[7] found the hydrolysis half-life time for commercial grade TRS to be 4.62 days at pH 4 and 25°C. Other half-life values were reported for different pH or temperature conditions than ours.[16,17] These apparent discrepancies in the reported half-life times could be due to differences in the types of buffer used or in the concentrations and the source of the herbicide (formulation, technical grade or analytical grade), thus making it difficult to compare the various studies directly.

Stability of triasulfuron in soil

The TRS degradation kinetics found during laboratory incubation in sterile and non-sterile soils are shown in Table 3. The degradation of TRS in the sterile and non-sterile Carmagnola soil followed first order kinetics ($r^2 > 0.981$). The degradation rate constant ($k \times 10^{-4}$) was 208 $days^{-1}$ in sterile soil and 219 $days^{-1}$ in non-sterile soil, and half-life values were 33.3 and 31.7 days, respectively. These two values were very similar and presented no significant differences ($P = 0.05$). Our results suggest that biological processes do not play a primary role in the degradation of TRS in this soil.

These results disagree with those of many authors who found for TRS lower half-life values in non-sterile soils as compared with autoclaved ones.[5,6,8] On the other hand Palm et al.[18] postulated that soil dissipation of rimsulfuron was mainly due to chemical hydrolysis.

As we believe that degradation in the soil is catalyzed by the soil organic and mineral colloids, it is possible that those researchers who autoclaved the soil altered this capacity. In effect, heat treatment determines a reduction in the surface area of the colloids and a solubilization of the exchange cations with a consequent reduction in interaction sites and a reduction in surface acidity. Treatment with ethylene oxide determines the formation of ethylene glycol which then interacts with some of the clays and the organic matter in the soil.[13,19] In some cases, this can determine an increase in pH and in the organic matter content of the soil. The sterilization performed on our soil samples did not determine a significant increase in pH (which increased from 7.9 to 8.0) neither was there an increase in organic

Table 2. Kinetics data of degradation of TRS at different pH values (mean of four replications, standard deviation <10%).

pH	$k_{obs} \times 10^{-4}$ (d^{-1})	*DT50 days*	*Correl. coeff.*
4	1633	4.2 (a)[a]	0.999
6	78	88.9 (b)	0.997
7	24	288.9 (c)	0.994
9	19	364.8 (d)	0.982

[a]DT50 values followed by the same letter are not significantly different ($P = 0.05$) as determined by analysis of variance (ANOVA) procedure.

Table 3. Kinetics data of degradation of TRS in different soils (mean of six replications, standard deviation <10%).

Soils	$k_{obs} \times 10^{-4}$ (d^{-1})	*DT50 days*	*Correl. coeff.*
Fossano	882	7.8 (a)[a]	0.988
Savigliano	609	11.3 (b)	0.991
Carmagnola non-sterile	219	31.7 (c)	0.995
Carmagnola sterile	208	33.3 (c)	0.981

[a]DT50 values followed by the same letter are not significantly different ($P = 0.05$) as determined by analysis of variance (ANOVA) procedure.

matter content (which was 0.95% and 0.94% in the sterile and non sterile soils respectively). This agreed with the results of Nègre et al.[13] who showed that the interaction of ethylene oxide with the soil is minimal when there is a low organic matter content and an alkaline pH.

TRS dissipated faster in acidic soil (half-lives = 7.8 and 11.3 days in Fassano and Savigliano soils, respectively) than in alkaline soil (half-life = 31.7 days in Carmagnola soil). These results are consistent with those recorded under laboratory conditions by Singh and Kulshrestha[6] and Dinelli et al.[7] where the rate of degradation increased with decreasing pH.

The slightly higher pH of Savigliano soil (6.3) as compared to that of Fossano (6.1) could be the cause of the greater half-life time obtained in the former. In fact, the calculation of the linear regression between the pH and half-life time values indicated a highly significant, positive correlation ($r^2 = 0.999$).

The results clearly show that TRS degraded faster in the three soils than in aqueous solutions, suggesting that soil catalysts contributed to the degradation of the herbicide. Clay minerals are considered the most active components in the abiotic decomposition of xenobiotics, due to the acidity of the hydratation water of the saturating cation and to the localization of the isomorphous substitutions in the clay structure. In a study on TRS interaction with smectites Pantani et al.[20] found a degradation product resulting from the hydrolysis of the herbicide on a clay surface. They observed that nontrite was more effective than hectorite in producing a metabolite at room temperature while the opposite occurred at a temperature of 50°C. They concluded that both the localization of the isomorphous substitutions and the water content of the clays affect the decomposing activity of clay minerals. In a further work, Pusino et al.[21] detected five TRS breakdown products in aqueous suspensions of Fe- and Al-rich montmorillonite demonstrating the active role played by the clay in the degradation of TRS.

Identification of breakdown products of trisulfuron

The main peaks of the mass spectrum of TRS (Fig. 1) were at m/z 402.2 ($M + H^+$), 423.8 ($M + Na^+$). The mass spectrum of product 1 (Fig. 2) exhibits a peak at m/z 236.1 corresponding to the sulfonamide moiety: 2-(2-chloroethoxy)benzenesulfonamide (CBSA), in agreement with Braschi et al.[15]. The peak at m/z 252.9 can be attributed to the $M + NH4^+$ ion. In both cases, the M+2 peaks (m/z 238.1 and 254.9) corresponding to the ^{37}Cl isotope are present (Fig. 2). In the same analytical conditions, product 2 was not detected probably because not ionizable.

The mass spectrum of product 2 (Fig. 3) obtained by GC-MS shows a peak at m/z 140 which agrees with the mass of the [(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino] (AMMT) produced by the cleavage of the sulfonylurea bridge.[15] The fragment at m/z 110 is obtained by the

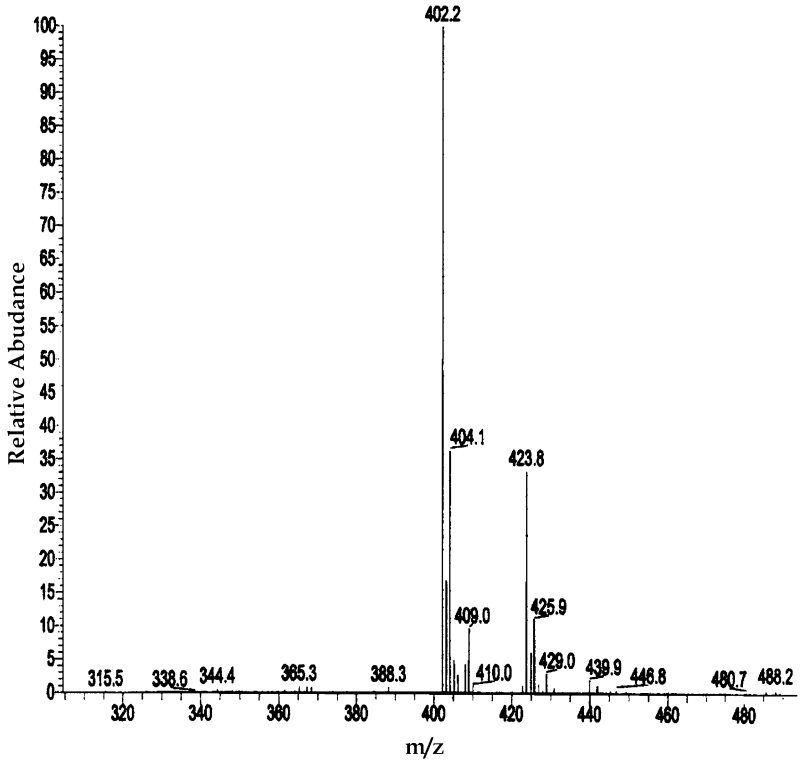

Fig. 1. Mass spectrum of TRS.

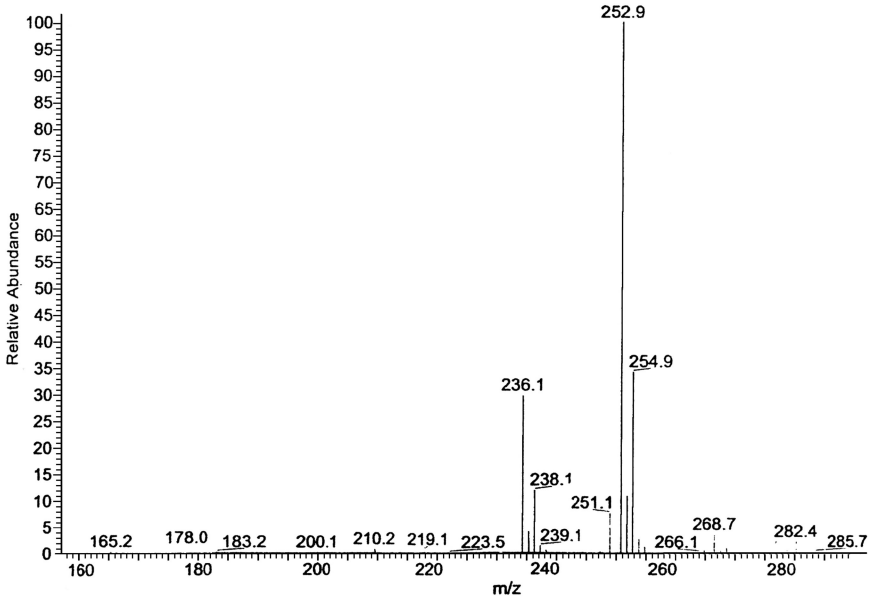

Fig. 2. Mass spectrum of CBSA.

elimination of the $-OCH_2$ group. The fragment at m/z 95 corresponds to the further elimination of the –NH group from the fragment at m/z 110.

The presence of CBSA and AMMT in the buffer solution at pH 4 confirms that the cleavage of the sulfonylurea bridge constitutes an important step in the degradation process, as was also demonstrated by Braschi et al.[15] This step, which corresponds to a hydrolysis mechanism, is the typical degradation pathway of sulfonylurea herbicides and also occurs in photolytical degradation.[2,22,23] Braschi et al.[15] also found [(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino] and 2-(2-chloroethoxy)benzenesulfonamide, to be the major breakdown components of TRS in buffer solutions with a pH in the 2–5 range. They observed a third breakdown product, identified as 2-(2-chloroethoxy)-N-[[(4-hydroxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl] benzenesulfonamide, which was unstable in acidic, aqueous solutions.

While a large amount of CBSA was isolated from the buffer solutions, only a small amount of AMMT was isolated. This agrees with the results of Braschi et al.[15] who found that the concentration of CBSA increased continuously over time, whereas AMMT degrades.

Stability of CBSA and AMMT in water

CBSA proved to be extremely stable over the whole range of pH conditions studied; it remained constant throughout the 95 days of the test (data not shown). Instead, AMMT underwent a very fast, chemical degradation, in particular at pH 4 and 9. At pH 4 the half-life time was 4.2 days while at pH 9 no trace of AMMT was to be found only 3 days after the beginning of the test. At pH 7 AMMT degraded slightly slower (half-life = 28.9 days) as compared to the acid and alkaline conditions, suggesting that hydrogenions and hydroxide ions have a catalytic effect on degradation (Table 4).

Strek[24] reported the degradation pathway of the triazine amine resulting from the hydrolysis of chlorsulfuron in a pH 5 buffer solution. In Strek's studies, the triazine amine

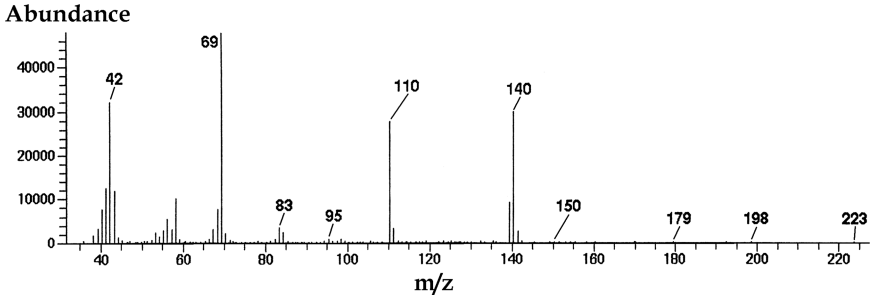

Fig. 3. Mass spectrum of AMMT.

Table 4. Degradation of AMMT in aqueous solutions at different pH (mean of four replications, standard deviation < 10%).

	AMMT remaining (%)		
Days	*pH 4*	*pH 7*	*pH 9*
0	100	100	100
3	61.6	n.d	0.0
7	33.3	91.0	0.0
20	5.3	68.3	nd
29	0.0	50.1	nd
72	0.0	19.0	nd

appeared to degrade due to the deamination, demethylation and subsequent hydrolytic cleavage of the triazine ring.

Stability of CBSA in soil

The degradation of CBSA in soil seems to follow a first-order kinetics; based on the first-order model, the estimated half-life value was 17.1 days ($r^2 = 0.983$) which is lower than that obtained for TRS in the same soil (31.7 days) (Fig. 4).

We are not aware of any works having been carried out on the degradation of sulfonamide in the soil. However, a study by Strek[24] on the degradation of (phenyl – U- ^{14}C) chlorsulfuron in the soil, showed that the sulfonamide is only slightly mineralized. In fact, after being incubated for 180 days only 5% of the radioactivity was recovered as ^{14}C carbon dioxide and bound residues.

Degradation of trisulfuron and CBSA by enriched cultures

As the test results on sterile and non-sterile soils demonstrated a non-biological degradation of TRS which did not agree with other works, it was necessary to increase our knowledge of the degradation activity of microorganisms on herbicides. Therefore, enrichment tests were set up with inocula of different origins.

Table 5. Degradation of TRS by mixed microbial coltures (mean of three replications, Standard Deviation < 10%). [a]: A = active sludges from a paper mill purification plant (active sludges 1); B = leacheale from a rubbish tip; C = active sludges from a sewage plant (active sludges 2); D = sediment from a rice-field which had previously been treated with another sulfonilurea (cinosulfuron); E = soil from an area previously treated with TRS.

Microbial colture[a]	*DT50*	*Correl. coeff.*	*pH range*
A	239 (b)[a]	0.965	6.7–6.8
B	231 (b)	0.979	6.7–6.8
C	112 (c)	0.903	6.1–6.6
D	298 (a)	0.976	7.0–7.2
E	121 (c)	0.982	6.5–6.7

[a]DT50 values followed by the same letter are not significantly different (P = 0.05) as determined by analysis of variance (ANOVA) procedure.

The degradation of TRS in mixed microbial culture was very slow and comparable to that found in sterile conditions at equivalent pH levels (Table 5). The addition of glucose did not increase the degradation rate of the herbicide even if it did induce a considerable increase in microbial activity (data not shown). This suggests that the TRS is extremely resistant to biodegradation if we consider both the great variety of microbial consortium examined and the fact that these came from environments which are subject to selective pressure as regards populations which are able to degrade synthetic molecules and/or complex structures. These results also agree with those obtained from degradation tests on sterile and non-sterile soil.

CBSA proved to be extremely stable in all culture medium, remaining constant for the 118-day duration of the test (data not shown). The results of the tests performed on CBSA show that this product is susceptible to degradation

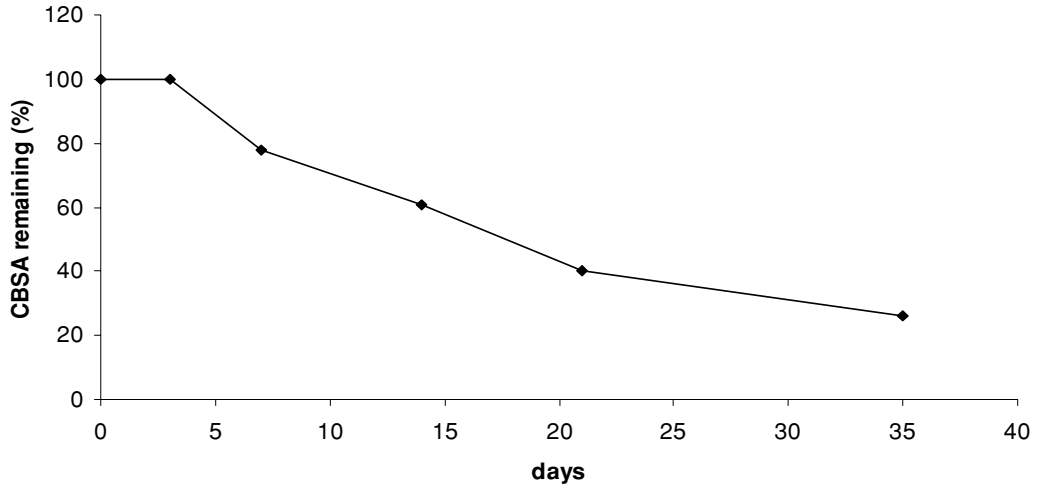

Fig. 4. Disappearance of CBSA in Carmagnola soil (mean of six replications, standard deviation <10%).

in the soil but only slightly chemically degradable. However, the degradation tests on enriched cultures indicated a certain resistance also to microbial degradation. This latter aspect is worth further investigation in order to clarify the degradation mechanism of the molecule in the soil. In any case, Strek[24] reported only minimal risk of acute toxicity from CBSA; it was also observed that neither pre- nor post-emergent application of 2000 g a.i. ha^{-1} CBSA adversely affected any of the species tested (soybean, rice, sorghum and spring wheat).

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