EFFECT OF TEMPERATURE ON DISSIPATION OF [^{14}C]-ATRAZINE IN A BRAZILIAN SOIL[1]

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ABSTRACT - The soil dissipation of the herbicide ^{14}C-atrazine was studied by solvent extraction, thin-layer chromatography and radiometric techniques. Results here presented show it was directly proportional to the temperature increases. As the temperature increased, less extractable and more bound residues were detected. At the end of the incubation period, soil extracts contained mainly atrazine but also its hydroxyderivative at 10°C and 20°C, and more hydroxyatrazine than atrazine at 30°C and 40°C. The calculated Arrhenius activation energy was very high (96 kJ . mol^{-1}) proving the predominance of chemical reactions favouring the hydrolysis. Exploratory analysis of the soil bound residues detected more than 90% as hydroxyatrazine, in all different temperature samples. Results suggest that in a soil with the characteristics of the soil here studied and at temperatures higher than 20°C, atrazine would not be a free contaminant because chemical degradation would result only in the non-phytotoxic hydroxyatrazine, either as available or as bound residues.

Index terms: dissipation, chemical degradation, residues, soil contamination.

EFEITO DA TEMPERATURA NA DISSIPAÇÃO DE [^{14}C]-ATRAZINA EM SOLO BRASILEIRO

RESUMO - A dissipação do herbicida ^{14}C-atrazina do solo foi estudada por extrações com solventes, cromatografia em camada delgada e técnicas radiométricas. Os resultados apresentados mostram que ela foi diretamente proporcional aos aumentos de temperatura. Quanto maior a temperatura, menos resíduos extraíveis e mais resíduos ligados foram detectados. Ao final dos períodos de incubação, os extratos de solo mantidos a 10°C e a 20°C continham atrazina mas também seu derivado hidroxi, e a 30°C e 40°C, mais hidroxiatrazina do que atrazina. A energia de ativação de Arrhenius calculada foi muito alta (96 kJ . mol^{-1}), provando a predominância de reações químicas que favoreceram a hidrólise. Análises exploratórias dos resíduos ligados ao solo detectaram mais de 90% como hidroxiatrazina, em todas as amostras de diferentes temperaturas. Os resultados sugerem que em solo com as características do aqui estudado e a temperaturas maiores que 20°C, a atrazina não seria um contaminante livre porque a degradação química resultaria somente no metabólito não fitotóxico hidroxiatrazina, ou como resíduo disponível ou como resíduo ligado.

Termos para indexação: dissipação, degradação química, resíduos, contaminação do solo.

INTRODUCTION

The herbicide atrazine is recommended for pre-emergence application in soil for crop plants as corn, sugar-cane, sorghum, soya, etc. (Esser et al., 1975), and it is pointed as the herbicide contaminant most found in ground water (Hance, 1987). In the soil, the herbicide undergo degradation by chemical and biological (mainly microbiological) activities

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(Hance, 1987). Kaufman & Kearney (1970) stated that chemical hydrolysis of chloro-s-triazines occurs faster than the microbial degradation resulting in the corresponding hydroxy-s-triazines. The hydrolysis is a detoxication reaction because hydroxyatrazine is a non-phytotoxic metabolite (Esser et al., 1975).

Thereafter, availability and bioactivity of herbicides in soil are also associated with the degradation process. Factors leading to production of non-phytotoxic substances are important to neutralize phytotoxic effects; but, a too fast degradation process may decrease the weed control (Reinhardt et al., 1990).

Some of the factors responsible for the hydrolysis of atrazine are soil pH (Hance, 1987; Reinhardt et al., 1990) and temperatures higher than 30°C (Kaufman & Kearney, 1970). Thus, as pointed by Walker & Welch (1989), weather conditions and edaphic factors should have influence on atrazine persistence.

Since Brazilian climatic conditions may reach temperatures as high as 40°C and soil pH is mostly acid, this study aimed to verify the behaviour of atrazine applied to soil incubated under temperatures ranging from 10°C to 40°C, for different periods of time.

MATERIAL AND METHODS

Herbicide

Commercial atrazine [2-chloro-4-(ethylamino)-6--(isopropylamino)-s-triazine] was supplied by CIBA--GEIGY S.A., Brazil, and analysed by the Chemical Section of the Instituto Biológico as 490.5 g . L^{-1} of atrazine. The corresponding radiolabelled, atrazine-ring--UL-[^{14}C], was obtained from Sigma Chemical Company, USA, with specific activity of 270 MBq . $mmol^{-1}$ (7.3 mCi . $mmol^{-1}$). Before using, the ^{14}C-atrazine 95% purity was checked by thin-layer chromatography (TLC) and radioscanner, as described bellow.

Soil

A Gley Humic soil sample, loamy textured (63% sand; 9.0% silt; 27.9% clay; 2.1% carbon, and pH (H_2O) 4.6), was collected at 0-30 cm depth from an agricultural area used for green stuff and corn crops in São Paulo State. The soil was air dried and passed through 2.0 mm sieve for homogenization.

Soil treatment

A 500 g soil sample was treated with a mixture of atrazine (2.18 mg) and [^{14}C]-atrazine (12.5 µCi) in 165 ml of water (equivalent to 33% of field capacity). The total ^{14}C applied was determined by combustion of (5x) 0.5 g soil samples (dry weight basis) in a Harvey Oxidizer OX-600. The $^{14}CO_2$ from soil combustion was trapped in 3 ml of ethanolamine in a liquid scintillation solution consisting of 4 g PPO, 0.2 g POPOP, 670 ml toluene and 330 ml Renex^R (Mesquita & Rüegg, 1984) plus methanol (6:4, scintillation solution:methanol) and quantified by liquid scintillation counting (LSC) in a Beckman LS-5801.

The remaining treated soil was then divided in equal portions of 140 g which were submitted to different incubation time and temperature treatments (Table 1). The soil water content was adjusted two times per week.

Degradation studies

Dissipation was studied by analytical determinations after ^{14}C-atrazine application to soil, for estimating the degradation process and products formed with time at different temperatures.

At each fixed incubation time (2x) 10 g of treated soil (dry weight basis) were Soxhlet extracted with 70 ml methanol for 8 hours. Extractable radioactivity was determined by LSC of (2x) 2 ml methanol aliquots of each replicate.

Another (2x) 15 ml methanol aliquots from soil extracts were concentrated by roto-evaporation (Büchi) at 55°C, the residue was redissolved in (3 x 1 ml) acetone which was chromatographed in TLC plates (silica gel-60 F_{254}). Developing system was firstly ethylether (15 cm) to separate atrazine and two of its metabolites (deisopropylatrazine and deethylatrazine). The plate was again unidimensionally developed in toluene:acetic acid:acetone (7:2:1) for 5 cm, to separate the hydroxyatrazine. The R_fs from standards of

TABLE 1. Soil treatments after herbicide addition.

Temperatura (°C)	Incubation time (days)				
10	20	40	70	100	140
20	20	40	60	80	100
30	10	20	30	50	70
40	10	20	30	40	50

hydroxyatrazine, deisopropylatrazine, deethylatrazine and atrazine were 0.16, 0.51, 0.57 and 0.68, respectively, determined under UV light (254 nm) and radioscanner in a TLC Scanner II LB 2723 (Berthold). The chromatograms were divided into strips and the silica gel was scrapped off for LSC.

Samples (2 x 0.5 g) of each extracted soil sample were combusted as described above, for determination of soil bound residues.

RESULTS AND DISCUSSION

Results on radiocarbon balance are presented in Table 2. The extractable residues decreased with time of incubation in all temperature treatments. The decrease was more pronounced as the temperature increased, as also reported by Rosário (1989). At 30°C, only 10 days were required for 71% recovery as extractable residues, but at 20°C and 10°C, 40 or 100 days respectively, were needed for the same recovery. The extractable residues detected at 20°C after 100 days (39%) were higher than the recovered after only 10 days at 40°C (30% - Table 2).

The amounts of bound residues increased with time in all temperatures, and the higher the temperature the highest the amount detected (Table 2). Only 10 days were enough for 26% and 55% of bound residues formation at 30°C and 40°C, respectively. At 10°C, only about 8% were detected as bound residues after 20 days, and thereafter the increase was slow, reaching 9.8% after 140 days. At 20°C, the increase of bound residues formation was more pronounced and 41% were detected after 100 days, in agreement with reports of Capriel et al. (1985), and Khan & Behki (1990). However, in samples incubated at 30°C, about half of the radiocarbon was detected as bound residues at the end of the 70 days, and most of the radiocarbon was detected as bound after only 50 days in samples kept at 40°C (Table 2).

Total recoveries generally decreased from the initial to the end of the experiment, under different temperatures, probably due to CO_2 evolution or volatilization with time (Kaufman & Kearney, 1970).

TLC of the soil extracts showed little degradation of atrazine to the phytotoxic deisopropylatrazine and deethylatrazine. Their amounts varied from 0.8% (at 40°C, 40 days) to 3.1% (at 20°C, 60 days), and from 0.1% (at 40°C, 40 days) to 3.7% (at 20°C, 20 days), respectively. However, the amounts of hydroxyatrazine and atrazine varied much more and were related with increases of temperature and incubation time (Figs. 1 and 2).

TABLE 2. Radiocarbon recoveries according to temperature treatment and time of incubation (in % of applied).

Temperature (°C)	Product[1]	Incubation time (days)									
		10	20	30	40	50	60	70	80	100	140
10	E	-[2]	84.3	-	86.5	-	-	78.6	-	73.1	61.7
	B	-	7.7	-	9.6	-	-	10.7	-	11.5	9.8
	T	-	92.0	-	96.1	-	-	89.3	-	84.6	71.5
20	E	-	83.4	-	71.2	-	59.7	-	51.4	39.1	-
	B	-	17.9	-	27.1	-	29.9	-	33.2	41.2	-
	T	-	101.3	-	98.3	-	89.6	-	84.5	80.3	-
30	E	70.7	57.1	49.3	-	40.1	-	33.2	-	-	-
	B	26.0	35.9	42.3	-	45.5	-	46.7	-	-	-
	T	96.7	93.0	91.6	-	85.6	-	79.9	-	-	-
40	E	30.1	23.8	15.1	16.8	19.5	-	-	-	-	-
	B	55.3	68.8	81.0	72.1	81.5	-	-	-	-	-
	T	85.4	92.6	96.1	88.9	101.0	-	-	-	-	-

[1] E = extractables; B = bound residues; T = total recovered.
[2] Not analysed.

Although amounts of extractable hydroxyatrazine did not exceed 23%, there was a slow increase with time in all different temperatures (Fig. 1). At 10°C, the hydroxyatrazine detected ranged from 1.2% (20 days) to 2.2% (140 days), while the amounts of atrazine decreased from 77% to 54% (Fig. 2) and the bound residues increased from 7.7% to 9.8% (Table 2) in the same period of time. At 20°C, the amounts of the hydroxy derivative increased from 5.0% (20 days) to 11% (100 days - Fig. 1), but atrazine recoveries decreased from 71.6% to 10.5%, in the same period (Fig. 2). The same 11% of hydroxyatrazine were detected within only 10 days under 30°C, and it increased until 22.7% (70 days - Fig. 1). The amounts of atrazine detected at 30°C (Fig. 2) varied from 54% (10 days) to 6.3% (70 days) and the bound residues formation increased from 26% to 47% in the same sampling times (Table 2). At 40°C, 16% were detected as hydroxyatrazine 10 days after the treatment, and thereafter it did not change very much (Fig. 1), but the amounts of atrazine decreased from about 12% to only 0.43% (Fig. 2). At the end of the experiments, the recovered products were as follow: atrazine>bound> hydroxyatrazine at 10°C; bound> atrazine = hydroxyatrazine at 20°C, and bound>hydroxyatrazine>atrazine at 30°C and 40°C.

Thus, the temperature treatments were highly and inversely related to extractable residue recoveries, but directly related to bound residues formation (Table 2) and degradation to hydroxyatrazine (Fig. 1).

The quickness of atrazine degradation followed a first order kinetics (Fig. 2). According to Walker (1987), this allows the calculation of half-lives and degradation rate constants at different temperatures, by the linear regression analysis of logarithms of the remaining concentration against time of incubation (Table 3). The calculation showed that each 10°C increase in the temperature decreased the half-life by a factor of, at least, 3.

Rate constant values were plotted against 1/Temperature (°K) and resulted in a straight line (Fig. 3) characterized by the Arrhenius equation,

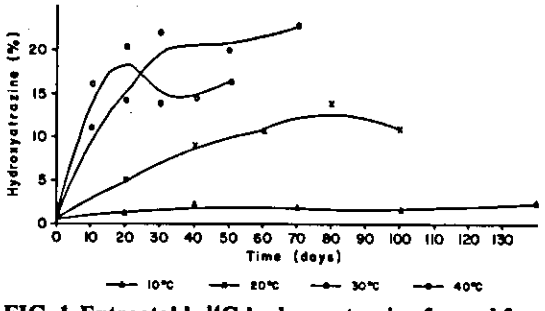

FIG. 1. Extractable ^{14}C-hydroxyatrazine formed from ^{14}C-atrazine applied to soil.

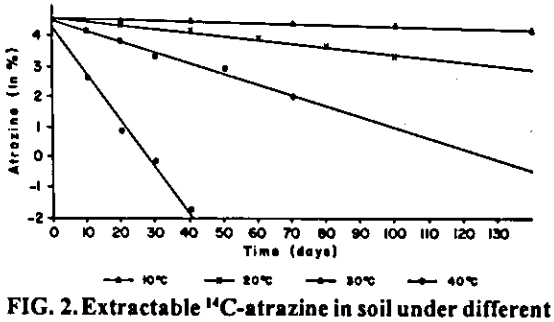

FIG. 2. Extractable ^{14}C-atrazine in soil under different temperatures.

TABLE 3. Degradation rate constants and half-lives of atrazine in soil at different temperatures.

Temperature (°C)	Rate constants (days-1)	Half-life (days)
10	0.00279	248.7
20	0.01223	56.7
30	0.03506	19.8
40	0.15350	4.5

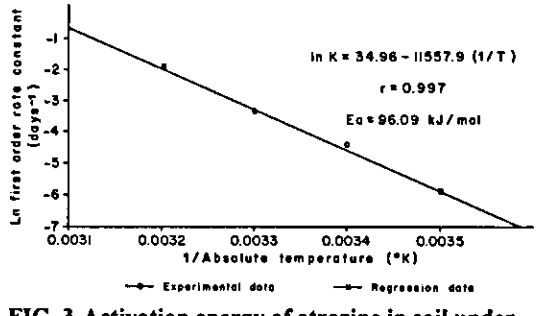

FIG. 3. Activation energy of atrazine in soil under different temperatures.

which results in the activation energy: ln K = ln A - - Ea/RT, where K is the degradation rate constant values, A is a soil constant which characterizes the dependence of half-life to temperature, R is the universal gas constant, Ea is the activation energy, and T is the temperature in °K (Walker & Brown, 1985; Walker, 1987; Rosário, 1989; Lehmann et al., 1993).

The calculated Arrehnius activation energy was about 96 kJ . mol^{-1}, which is very high compared with other herbicides and soils (Walker & Brown, 1983; Walker, 1987; Vega et al., 1992), and is characteristic of predominance of chemical reactions for degradation of atrazine (Vega et al., 1992). This high value is due to the temperature effect, but may also be associated with the low soil pH, favouring atrazine hydrolysis to the hydroxy derivative detected in these experiments even at temperatures of 10°C and 20°C (Hance, 1987; Reinhardt et al., 1990; Reinhardt & Nel, 1993).

The characterization of some samples of soil bound residues performed at the Centre for Land & Biological Resources (Agriculture Canada) is presented in Table 4. Only hydroxyatrazine and atrazine were detected and no evidence was obtained for the presence of any dealkylated toxic products. Hydroxyatrazine represented more than 90% of the bound residues in all analysed samples.

Therefore, atrazine is not a probable free contaminant for crops in soils with the characteristics of the soil here studied and suitable water content under the warm Brazilian weather conditions, because the predominance of chemical reactions resulted mainly in the non-phytotoxic hydroxyatrazine.

TABLE 4. Recovery of soil bound residues from ^{14}C-atrazine.

Sample	Bound residues (% of applied)	OH-atraz.	Atraz.
		μg/g	
10°C, 140 days	9.8	0.46	0.042
20°C, 100 days	41.2	0.96	0.132
30°C, 70 days	46.7	1.64	0.172
40°C, 50 days	81.5	2.97	0.242

CONCLUSIONS

1. The availability of atrazine in the soil solution decreases with time independently of the temperature.

2. The higher the temperature less extractable and more bound residues are detected.

3. Degradation of atrazine in hydroxyatrazine is related to increases in temperature and incubation time, and the process involved is mainly chemical degradation.

4. The half-life of atrazine in soil decreases with increases in temperature.

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