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Atrazine in Water and Biodegradation in a Recharge Area of Guarany Aquifer in Brazil

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The region of Ribeirao Preto City (Figure 1), located in Sao Paulo State, southeastern Brazil, is an important sugarcane, soybean and corn producing area. This region is also an important recharge area for groundwater of the Guarany aquifer, a water supply source of the city and region. It has an intercontinental extension that comprises areas of eight Brazilian states, as well as significant portions of other South American countries like Argentina, Uruguay, and Paraguay, with a total area of approximately 1,200,000 Km^2.

Intensive cultivation in this area has required the constant and sometimes abusive use of pre-emergent herbicides and fertilizers. The risk of groundwater contamination by those chemicals, which are normally reapplied annually, has been a major concern. Due to the high permeability of some soils present in this region, the high mobility of the herbicides and fertilizers applied, and being a recharge area, it is important to investigate the potential transport of applied herbicides to underlying aquifer.

Although it was generally accepted that pesticides would not leach to groundwater, recent studies indicate agrichemical leaching as an important source of agricultural non-point-source pollution (Smith et al. 2001), particularly in the last decade (Bouwer 1990). Other studies have indicated that some American aquifers were contaminated with both inorganic and organic compounds, some of which were pesticides (Williams et al. 1988).

Currently, most of the sugar cane crop grown in the area is burned to help harvesting. However, this practice can alter soil properties. There is a new trend toward the use of mechanical harvesting without burning that allows the straw to more readily decompose in soil. This would maintain a better soil structure and moisture, but could interfere with the leaching of solutes. Sometimes, peanuts are grown in rotation with sugarcane. Triazine herbicides such as atrazine, ametryn, and simazine are used in the area and are known to have high potential for groundwater contamination (Cerdeira et al. 2000).

The cultivation of grain and sugar cane in this area demands the constant use of the herbicide atrazine (2-chloro-4-(ethylamino)-6-(isopropylamino)-S-triazine) that is a

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Figure 1. Map of South America showing the city of Ribeirao Preto, Brazil, where the watershed is located.

selective triazine herbicide commonly used to control broadleaf and grassy weeds in corn, sorghum, sugarcane, pineapple, and other crops (EXTOXNET 1988). In sugarcane, atrazina herbicide is applied once a year, every crop season, at the rate of 3.0 Kg/ha (Kilogram per hectare) of active ingredient. Atrazine is relatively persistent in soil and is moderately to highly mobile in soils with low clay or organic matter content. Because of those characteristics it is not adsorbed strongly to soil particles and has a relatively long half-life (60 to 100 days). Wauchope (1987) has shown that it has a high potential for groundwater contamination despite its moderate solubility, which explains the detection of the pesticide in concentrations that exceed the health advisory level in some wells in the United States located on irrigated lands (Belluck et al. 1991). A recent law from the Brazilian Health Ministry has set the maximum concentrations in drinking water to 10.0 mg/L (ppm) of nitrates and only 2.0 ug/L (ppb) of atrazine and simazine (Ministerio da Saude 2000).

Although there has been some research on groundwater contamination with triazines in Brazil, none of the studies have been conducted to determine the precise amounts of triazines present in this aquifer (Cerdeira et al. 2000). Some studies of atrazine biodegradition in the area have been conducted (Ueta et al. 1999). This paper reports research conducted to determine precise concentrations in the Guarany Aquifer as well as atrazine biodegradation in soils of the area.

MATERIALS AND METHODS

The study area is located at Espraiado watershed, (4,000 ha), north of the São Paulo State, Brazil, in the counties of Ribeirao Preto, Cravinhos, and Serrana with

the geographical coordinates 21°05', 21°20' south and 47°40', 47°50' west. The climate of the area is tropical with dry winter savanna, an annual median temperature around 21°C and rainfall varying between 1300 and 1500 mm/year. The main soils are classified as Clayey Eutroferric Red Latosol (LVefb), Psamitic Distrofic Red Latosol (LVdq) and Quartzarenic Neosol (RQ), according to the Brazilian System of Soil Classification (EMBRAPA 1999).

Nine surface water sample points were selected in the Espraiado stream in the watershed, during the years of 1995-1998. Samples were collected in the months of October, November, December, January, March, May, and July of each year. Four replications were collected at each site for a total of 252 surface water samples per year. Groundwater was also collected during the same months from county groundwater wells located at the edge of the watershed during the years of 1999 and 2002. The following seven wells were studied: Palmares, Sao Jose, Sao Sebastiao Velho, JP, Higienopolis, Schimidt, Jardim Recreio, and DAERP Central. The water samples (1-liter) were stored in amber flasks and kept at 4°C until analysis using the following HPLC (High Performance Liquid Chromatography) procedure: A standard solution of atrazine (100%, Supelco) was prepared in HPLC-grade methanol (Merck) at a concentration of 1.0 mg/ml. Working solutions at concentrations of 0.08, 0.20, 0.32, and 0.40 μg/ml, were prepared by appropriate dilution. A caffeine solution (used as the internal standard) for the confirmation of herbicide residues by GC-MS was prepared in methanol at a concentration of 5.0 μg/ml.

HPLC analyses were performed with a Shimadzu liquid chromatograph (Kyoto, Japan) consisting of an LC-10AD pump, a UV detector (SPD-10AV) operating at 220 nm, an automatic injector (SIL 10A) with a 100 μl loop and a Chromatopac C-R6A integrator. The presence of atrazine in water samples was confirmed using a Shimadzu GC-MS system model QP5000 (Kyoto, Japan) that consists of a gas chromatograph equipped with a split/splitless injector (t_V = 240°C, splitless, 0.75 min sampling time) and coupled to a mass selective detector operating in the SIM mode. The calibration curves were obtained by spiking 100 mL aliquots of water purified in a MILLI Q®-plus system (Millipore) with 25.0 μL of each standard solution, resulting in concentrations of 0.02 to 0.1 μg/L water. In the GC-MS analysis the water samples were also spiked with 25.0 μL of internal standard solution, caffeine 5 μg/mL (Lanchote et al. 2000).

To predict the atrazine leaching in the area, the CMLS-94, Chemical Movement Layered Soil, (Nofziger and Hornsby 1994) simulation model was used. Data obtained by the simulations were then evaluated with those of depths of the groundwater levels. The input data used were: a) sugar cane cultural coefficient (Kc) (Paranhos 1987); b) soil type by levels: percent of organic carbon, density (Mg m^{-3}), volumetric content of water (%), field capacity, wilting point, and saturation; c) weather: daily maximum and minimum temperatures, rainfall and

evaporation, for a period of four years; d) atrazine properties: K_{OC} and half life ($t_{1/2}$). Different simulation scenarios were made to evaluate the atrazine movement in the vertical profile of Clayey Eutroferric Red Latosol (LVefb), Psamitic Distrofic Red Latosol (LVdfq), and Quartzarenic Neosol (RQ).

Soil samples were collected monthly during the period of February 1996 to February 1997 from Espraiado watershed area for general microbiological and atrazine biodegradation studies (Ueta et al. 1999). Soil samples (zero to 20, and 20 to 40 cm. depths) representing nine different points were collected monthly. Soil suspensions, 5.0g of each soil sample in 50.0 mL of sterile water were agitated in a rotary shaker for 3h at 30°C. After shaking, aliquots of 8.0 mL were transferred to a glass tube containing 40.0 mL of tem-fold diluted Yeast Nitrogen Base (YNB) liquid medium with 1mg/mL atrazine, 2,4-D (2,4-Dichlorophenoxyacetic Acid) to promote more diversity, and control. After 21-day incubation, aliquots of soil suspensions in glycerol (10%) were prepared and kept in the freezer (-20° C) as source of microbial population for microbiological and biodegradation studies. For this particular study of atrazine biodegradation, two sets of experiments were done with a few selected samples. In the first one, 100 μL of six frozen suspensions were transferred to tubes containing 5.0 mL of trypticase medium, and incubated for up to 48 h at 30°C to promote general microbial growth. Twenty μL of the trypticase culture were then inoculated in 3.0 mL cetrimide medium (a selective medium prepared from cetrimide agar to promote growth of pseudomona-like microorganisms, Merck) at 35°C for 48 h. For the atrazine biodegradation assay, aliquots of 500 μL of the cultures in trypticase or 800 μL of the cultures in cetrimide were centrifuged and washed for removal of the medium. Centrifuged cells were incubated for 26 days in 5.0 mL of medium at pH 6.8, containing $MgSO_4$ (0.5g/L), NaCl (0.1g/L), $CaCl_2$ (0.1g/L) and KH_2PO_4 (1.0g/L) with $(NH_4)2SO4$ at 5.0g/L (MMA, minimum medium with ammonium sulfate as source of N) or without it (MM). Atrazine was added at the final concentration of 10.0 μg/mL. Corresponding control cultures were included.

In another set of experiments for atrazine biodegradation, 100 μL of ten frozen glycerol soil suspensions were incubated in trypticase medium containing 50ug/mL atrazine for 26 days to assess biodegradation. Controls without microorganisms were run. In both experiments, after extraction, atrazine was measured by HPLC, as described.

RESULTS AND DISCUSSION

Results from water samples collected at two month intervals in the Espraiado stream at the watershed, during the years of 1995-1998, and from six municipal groundwater wells located at the edge of the watershed during the years of 1999 and 2001, showed only four atrazine detections in surface water in the year 1996 with residues varying from 0.02 to 0.09 ppb. However, none of them were confirmed with GC-MS. No atrazine was detected in groundwater samples.

The results obtained by the CMLS-94 simulations predicted that atrazine, after four years from the application date, would not have reached the depth of the confined aquifer (40m). However, as a non-confined more superficial water table exists in the study area (with depths varying between zero and 20 m) it was shown that there is a risk of the herbicide reaching the aquifer (Table 1).

Since the half-life ($t_{1/2}$) of atrazine is highly influenced by the soil pH and by organic matter content (Walker and Blacklow 1994), also Quartzarenic Neosol (RQ) has pH values varying from 7.3 at 0-50 cm to 7.0 at 50-60 cm (Cerdeira et al. 2000), those characteristics would favor the mobilization of the atrazine molecules and result in leaching to greater depths (Table 1). In Clayey Eutroferric Red Latosol (LVefb) and Psamitic Distrofic Red Latosol (LVdq), the respective values of pH remained acidic and favored a little movement of atrazine in those soils (Table 1). In that situation, the final amount projected by simulation scenarios was mainly influenced by t½ values of atrazine in the respective soil type. Atrazine has shown no potential to reach groundwater when evaluated by the CMLS-94. This result agrees with the information obtained by means of monitoring wells located in the study area, where atrazine was not detected in the water.

Table 1. Partition Coefficient (Koc), half-life (t½) of atrazine, depth values (DPT, m) and amount (AMT, kg/ha) reached at the end of simulations for each type of soil.

SOIL TYPE	ATRAZINE			
	characteristics		movement	
	Koc(L/kg)	t½(days)	DPT	AMT
LVdq (Psamitic Distrofic Red Latosol)	224.3	54	1.67	9.2×10^{-7}
Lvefb (Clayey Eutroferric Red Latosol)	187.1	262	1.43	1.4×10^{-1}
RQ (Quartzarenic Neosol)	305.7	181	2.88	3.4×10^{-2}

For atrazine biodegradation studies, six soil suspensions were selected: three atrazine-treated, two treated with 2,4-D to promote more diversity and one without any herbicide addition. After incubation in trypticase medium, to promote general microbial growth, with or without transfer to cetrimide medium, to promote growth of pseudomona-like microorganisms, the cultures were used to evaluate the ability of the microbial population to biodegrade atrazine (10 μg /mL) in minimum medium without (MM) and with ammonium sulfate (MMA) as the source of N.

After a 26-day of soil and atrazine incubation period, samples for atrazine determination were collected. Results (Table 2) have shown that two of the six samples, number 4 and 5, were able to degrade totally the atrazine in the medium without N source, but they remained intact in MMA medium. In one sample, number 1, 76% of the atrazine disappeared from the MMA medium, but not from

MM. Cultures from the other three samples, number 2, 3, and 6, have shown no atrazine consumption in MM or MMA media. The incubation of the cells in cetrimide, a selective medium used to promote growth of pseudomona-like microorganisms, did not allow microbial growth in samples 2 and 4. The biodegradation assays with cetrimide-incubated cultures resulted in 80% atrazine disappearance in one sample, number 6, in MM medium. These results have shown atrazine biodegradation with large variation depending on soil and media conditions (Table 2).

Table 2. Percentage of atrazine biodegradation detected in six soil suspension samples after 26-day incubation in MMA, minimum medium with ammonium sulfate as source of N or without it, MM, cetrimide alone, MM and MMA with cetrimide, MMcet and MMAcet, respectively. Soil samples varied from Clayey Eutroferric Red Latosol (LVefb), Psamitic Distrofic Red Latosol (LVdfq), and Quartzarenic Neosol (RQ).

SOIL SAMPLES	MM	MMA	Growth in cetrimide	MMcet	MMA cet
1	0	76	+	0	0
2	0	0	-	0	0
3	0	0	+	0	0
4	100	0	-	0	0
5	100	0	+	0	0
6	0	0	+	80	0

In an attempt to improve the atrazine biodegradation rates by microorganisms, experiments were done in MM and MMA media with suspensions incubated in trypticase for 48 h, transferred or not transferred in cetrimide medium. For the incubation period of seven to 30 days, no degradation was detected, (data not shown).

Another experiment was performed using ten frozen samples of glycerol soil suspensions in trypticase medium with 50 ug/mL of atrazine following the procedure previously described (see Material and Methods). Disappearance of this atrazine from trypticase medium was shown in nine out of 10 soil suspensions, one of them with 82% atrazine (50 ug/mL) reduction, as shown in Table 3.

The results obtained with the microbial populations present in the soil samples incubated in the laboratory with atrazine have shown the presence of atrazine biodegraders. The initial incubation of soil samples with atrazine is not a decisive factor on the selection of metabolizing strains. The assay conditions used to demonstrate the disappearance of atrazine from the minimal or even rich medium are highly relevant. In both experiments with soil suspensions, using poor (MM or MMA) or rich (Trypticase) media it was shown atrazine biodegradation accomplished by strains with different characteristics. It can be concluded that microbial species involved in the biodegradation are different, not necessarily

demanding the presence of a nitrogen source. It was only in 1994 (Yanze-Kontchou and Gschwind 1994), after three decades of searching, that atrazine degradation by a single species was described. Studies with microbial population in soils of the area and the atrazine biodegradation ability of these communities have identified some strains isolated under atrazine selective conditions. One of these studies resulted in species identified as *Rhodotorula rubra, Candida sp., Serratia marscescens,* and *Klebisiella pneumoniae* (Bombonato 2001).

Table 3. Percentage of atrazine (At) reduction in soil samples incubated 26 days in trypticase medium at 30°C. Soil samples varied from Clayey Eutroferric Red Latosol (LVefb), Psamitic Distrofic Red Latosol (LVdfq), and Quartzarenic Neosol (RQ).

SOIL SAMPLES	%At REDUCTION
1	74
2	50
3	54
4	57
5	68
6	53
7	82
8	0
9	50
10	23

The detection of the presence of atrazine biodegrading microorganisms may explain the absence of atrazine residues in groundwater at the edge of the watershed, as shown in the laboratory residue analysis and in the simulation model.

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