Determination of ametryn herbicide by bioassay and gas chromatography-mass spectrometry in analysis of residues in drinking water

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Pervenuto in Redazione

Um método biológico simples, rápido e quantitativo para a análise de ametrina em águas superficiais e subterrâneas foi comparado a um por cromatografia gasosa com detetor de massas (CG/MS). Este método foi baseado na atividade da ametrina em inibir o crescimento das radículas das sementes de Lactuca sativa L.O procedimento apresentou sensibilidade de 0.01µg/L e foi aplicado até a concentrações de 0.6µg/L. Esterilização, seleção prévia das sementes e equipamentos especiais não foram requeridos. Desta forma concluimos que o método biológico apresenta sensibilidade compatível com o cromatográfico (CG/MS). No entanto a baixa correlação entre os métodos sugere o bioensaio apenas como método de triagem na avaliação de resíduos de ametrina em água.

ABSTRACT

A simple, rapid, and quantitative bioassay method was compared to a gas chromatography /mass spectrometry (GC/MS) procedure for the analysis of ametryn in surface and groundwater. This method was based on the activity of ametryn in inhibiting the growth of the primary root and shoot of germinating lettuce, *Lactuca sativa L.* seed. The procedure was sensitive to 0.01 ug/l and was applicable from this concentration up to 0.6 µg/l. Initial surface sterilization of the seed, selection of pregerminated seed of certain root lengths, and special equipment are not necessary. So, we concluded that the sensitivity of the bioassay method is compatible with the chromatographic method (GC-MS). However, the study of the correlation between methods suggests that the bioassay should be used only as a screening technique for the evaluation of ametryn residues in water.

RIASSUNTO

Un metodo biologico semplice, rapido e quantitativo per saggiare la presenza di ametrina nelle acque di superficie e di falda è stato messo a confronto con la gascromatografia e la spettrometria di massa. Questo metodo si basa sulla capacit' dell'ametrina di inibire la crescita delle radici primarie e del germoglio della lattuga (Lactuca sativa L.). Il metodo è sensibile a partire da 0.01 µg/l fino a 0.6 µg/l. Non è necessario sterilizzare la superficie del seme, selezionare seme con determinate lunghezza della radice e non è necessario alcun strumento particolare. La combinazione del metodo biologico con la gascromatografia può rappresentare un sistema di saggio della presenza della ametrina comodo e poco costoso per monitoraggi delle acque su larga scala.

KEY WORDS: Bioassay; CG/MS; Ametryn herbicide residues; Water

INTRODUCTION

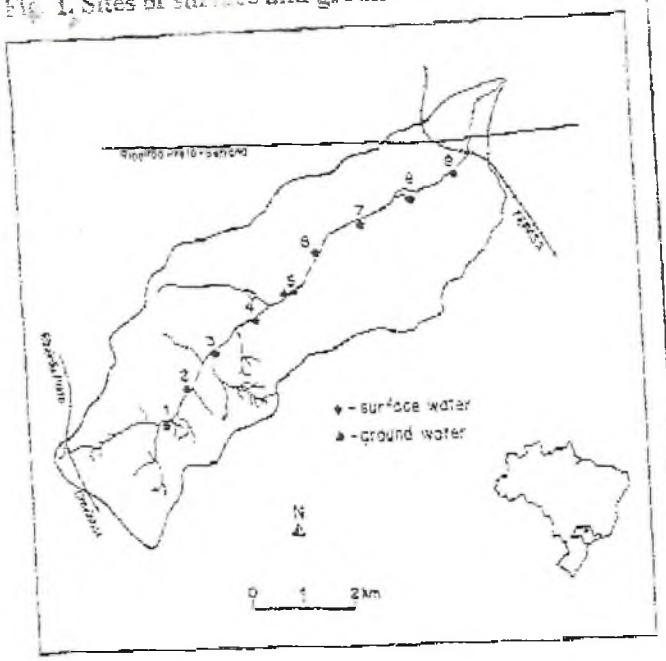
The beneficial effects of herbicides are sometimes affected by their persistence in the environment. Water may be contaminated by herbicides from aerial spraying, run off from land treated by direct introduction of herbicides into water to control aquatic weeds, and leaching to groundwater. The availability of a monitoring system for herbicides, particularly in flowing waters such as irrigation canals is essential so that damage to the environment, crop plants through the use of contaminated water, and human health can be avoided [13, 15, 16]. Ametryn, a member of the triazine family, is a herbicide which is used to control broadleaf weeds and annual grasses in pineapple, sugarcane and bananas. It is used on corn and potato crops for general weed control [17]. Ametryn's half-life in soil , the amount of time it takes to degrade to half of the original concentration, is 70 to 250 days, depending on the soil type and weather conditions. Degradation from the soil is principally by microbial process. Ametryn moves both vertically and laterally in soil due to its high water solubility. Because it is persistent, it may leach as a result of high rainfall, floods, and furrow irrigation [4, 14, 17]. Ametryn is slightly toxic to humans. Simptoms of acute exposure to high doses include nausea, vomiting, diarrhea, muscle weakness, and salivation. Ametryn is moderately irritating to the eyes, skin, and respiratory tract [14]. Triazines as selective herbicides were introduced about 30 years ago. Determination of the movement, leaching, and residual properties of herbicides in water can be accomplished by the use of analytical [5, 8, 20] or bioassay [7, 8, 10, 11, 12] techniques. In the present study, we reported the application of bioassay and GC/MS methods for the analysis of ametryn resides in surface and ground water from the Espraiado Stream watershed located southeast of the town of Ribeirão Preto, SP, Brazil. This watershed is located in a region of intensive sugar cane monoculture, the third culture in the country in terms of pesticide application and because it represents one of the recharging points of the water table of Botucatu aquifer, the largest and the most important one in the center-south region of Brazil, including eight Brazilian states and parts of Argentina, Uruguay and Paraguay [9].

MATERIAL AND METHODS

Water Samples

Surface water samples (1 liter) were collected from the Espraido watershed (Ribeirão Preto, SP, Brazil - Figure 1) during the period from October 1995 to July 1996, at

Fig. 1. Sites of surface and ground waters sampling



nine different points. Groundwater samples from sites near the river were collected during the same period. The water samples were stored in amber flasks and kept at 4°C until analysis.

Reagents

The standard solutions of ametryn (100%, Supelco, Inc., Bellefonte, PA, USA) was prepared in chromatography grade methanol (EM Science, Gibbstown, NJ, USA) at respective concentrations of 0.5, 1.0 and 1.0 mg/mL. The stock solutions were used to prepare dilutions at concentrations of 0.08, 0.2 and 0.4 µg/mL.
The caffeine solution used as internal standard was prepared in methanol at the concentration of 5 µg/mL. The 4 M sodium hydroxide solution was prepared in water purified with the Milli Q® system (Millipore, São Paulo, Brazil) and washed with dichloromethane: isopropanol (9:1, v/v). Chromatography grade ethyl acetate (EM Science, Mallinckrodt, Phillipsburg, NJ, USA) used for the extraction of ametryn from the water samples was purified by distillation.

GC/MS conditions

The presence of ametryn in water samples was confirmed using a Shimadzu GC-MS system model QP5000 (Kioto, Japan) consisting of a gas chromatography equipped with a split/splitless injector (t =240°C, splitless, 0.75 min sampling time . Selective detector operating in the SIM mode (electronic impact, 70ev). The ametryn was separated on a 0.25 mm x 30 m DB-5 capillary column, with 0.25 µm film thickness (J&W Scientific, Folson, CA, USA). The column was maintained at 60°C for 1min and then heated at 20°C/min up to 150°C and at 10°C/min up to 280°C. Helium was used as the mobile phase, at a total flow rate of 28 ml/min. Monitored ions: ametryn: 227, 212; caffeine 194, 109; simazine: 201, 186; [illegible] atrazine: 215, 200, 173.

Sample preparation

The extraction procedure consisted of the addition of 100 ml of water samples, previously filtered through 0.22 µm membranes (Millipore, São Paulo, Brazil) to remove material in suspension. After the addition of 25 µL of the 4 M NaOH solution supplemented with 25 µL of internal standard solution (caffeine, 5 ug/ml), the samples were extracted with 15 ml of ethyl acetate. After shaking for one hour, the organic phases were transferred to test tubes and centrifuged at 1800 g for 5 min for full separation of the aqueous phase. Volumes of 5 mL of the organic layers were transferred to conic tubes and evaporated dryness under a nitrogen flow, at the temperature of 35OC. The residues were dissolved in 25 µL acetone, residue analysis grade (EM Science) and 2 µl were chromatographed under the conditions described above.

Calibration curves

The calibration curves were obtained by spiking 100 ml aliquots of water purified in a MILLI Q®-plus system (Millipore, São Paulo, Brazil) with 25 µl of each standard solution, resulting in concentrations of 0.02 to 0.1 µl/l water. Inthe CG-MS analysis the water samples were also spiked with 25 µl of internal standard solution (caffeine 5 µg/ml).

Bioassay

The *Lactuca sativa L.* seed used was a commercially available variety, Casa Massaro Sementes Ltda, Ribeirão Preto, SP, Brazil. Whatman n. 20 filter paper disks were placed on 10 x 1.5 cm Petri dishes and soaked in 4.0 µL of water samples in quintuplicate. Ten seeds were placed on each plate and maintained at 25°C for 72 hours, with 80-90% relative air humidity and a 16 hour photoperiod (approximately 3800 lux). The ametryn concentrations in the samples were evaluated on the basis of inhibition of rootlet growth in *Lactuca sativa L.* [5] seedlings.
Solutions of ametryn of unknown concentration, may be subjected to the procedure herein described, and growth inhibition may be expressed in percentage of control. This may then be compared with the standard curve, and the presumptive concentration of ametryn may be thereby ascertained.

Calibration curve

The calibration curve was obtained by spiking 100 ml aliquots of water purified in a Milli Q®-plus system with 25 µl of each standard solution, resulting in concentrations of 0.01 to 0.6 µg/l. The calibration curve was constructed by plotting the percentages of rootlet growth in relation to the control on the ordinate, in a probability scale, and the ametryn concentrations (µg/L) on the abscissa .

RESULTS AND DISCUSSION

The calibration curve presented in Figure 2 shows that the inhibition of *Lactuca sativa L.* rootlet growth was linear in the concentration range of ametryn in water of 0.01-0.6 µg/L. The data presented in Table I suggested the occurrence of stimulation of rootlet growth in some samples. Comparison of the ametryn concentrations in water samples analyzed by GC-MS and by bioassay (Figure 3) demonstrates results of the same order of magnitude but the low correlation coefficient (r = 0.367) suggests that the bioassay should be used only as a screening technique in the evaluation of water contamination by ametryn residues. It should be emphasized that the bioassay method, despite its low specificity, presents the advantage of detecting residues at concentrations below the Maximum Admissi-

Fig. 2. Bioassay calibration curve for ametryn concentrations of 0.01 - 0.6 µg/l. Ract grouth was plated on probability scale against ametryn concentration in water (y = 1.42 - 2.08x; r = 0.752)

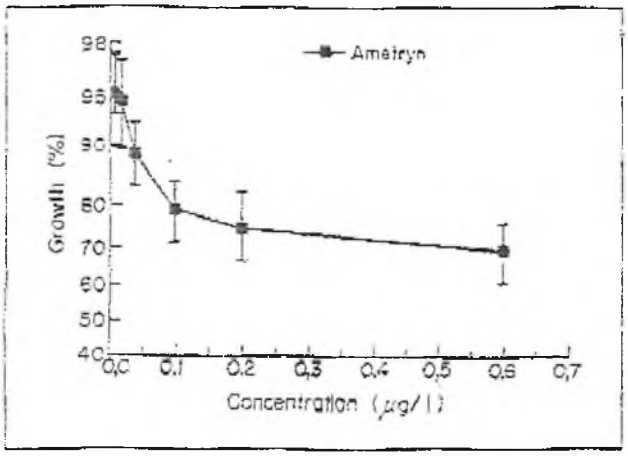


Fig. 3. Ametryn concentration in surface water determined by bioassay and CG-MS (y = 0.09 - 1.49x; r = 0.367)

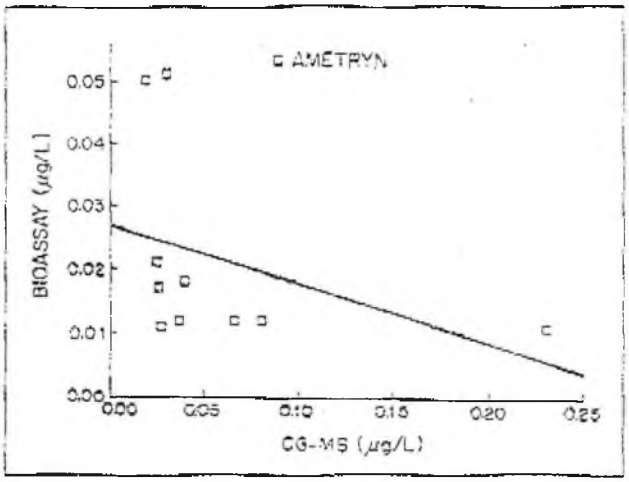


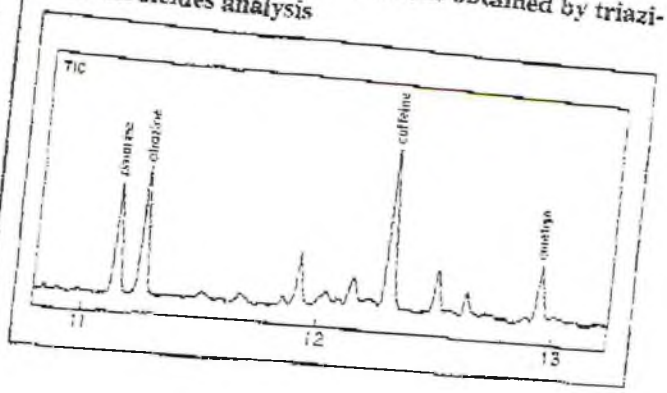
Table I. Ametryn concentration determined by bioassay in water samples

Sample	Date collected	Ract grouth (mm) mean ± SD	Ract grouth percent of control (%)	Ametryn concentration (µg/L)
1B	15/01/96	2.75 ± 0.35	135.7	ND
2C	13/12/95	2.02 ± 0.18	99.7	0.01
9A	13/12/95	1.46 ± 0.20	72.0	0.40
4B	13/12/95	1.77 ± 0.24	87.4	0.05
4D	15/01/96	1.93 ± 0.34	95.3	0.02
9B	19/03/96	2.19 ± 0.20	108.0	ND
7B	15/01/96	2.00 ± 0.16	98.7	0.01
1A	13/12/95	2.58 ± 0.30	129.0	ND
4D	13/12/95	2.40 ± 0.25	120.0	ND
3D	13/12/95	2.00 ± 0.12	98.7	0.01
3C	15/01/96	2.87 ± 0.33	143.5	ND
7B	13/12/95	2.00 ± 0.27	98.7	0.01
4B	19/03/96	2.01 ± 0.19	99.2	0.01
3B	31/07/96	2.45 ± 0.22	122.5	ND
2D	15/01/96	1.85 ± 0.23	92.5	0.021
5A	15/01/96	2.40 ± 0.20	120.0	ND

ND = not detected in concentration equal or above 0.01 µg/L

the Limit of 0.1 μg/L [4] being of easy execution and permitting screening in laboratories that are not equipped for residue analysis by chromatographic methods. As Banks and Merkle [3], we found out that bioassay techniques were more limited in range and more variable than gas chromatography. Many herbicides can stimulate plant growth at subletal rates [21], what have introduced further difficulties into quantitative measurements. Furthermore, the response of the bioassay plant does not differentiate between the original compound and its bioactive derivatives. Otherwise, the analytical method is time consuming and requires the use of expensive and sofisticated instruments. So, we concluded that the sensitivity of the bioassay method is compatible with the chromatographic method (GC-MS) for the analysis of ametryn residues in water. However, the study of the correlation between methods suggests that the bioassay should be used only as a screening technique for the evaluation of ametryn residues in water.

Fig. 4. Chromatogram of total ions obtained by triazinics herbicides analysis



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