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Rapid estimation of readily leachable triazine residues in soils using automatic kinetic bioaccessibility assays followed by on-line sorptive clean-up as a front-end to liquid chromatography

Ana C.F. Vida [a], David J. Cocovi-Solberg [b], Elias A.G. Zagatto [a], Manuel Miró [b,*]

[a] Center for Nuclear Energy in Agriculture, University of Sao Paulo, Avenida Centenário 303, 13416-000 Piracicaba, Brazil
[b] FI-TRACE group, Department of Chemistry, University of the Balearic Islands, Carretera de Valldemossa km 7.5, E-07122 Palma de Mallorca, Spain

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ABSTRACT

An automatic batchwise bioaccessibility test was proposed for on-line monitoring of readily mobile pools of ametryn and atrazine residues in agricultural soils with different physicochemical properties. A 0.01 mol L^{-1} $CaCl_2$ solution mimicking rainwater percolation through the soil profiles was used for the herbicide extractions. The extract aliquots were successively sampled at regular time intervals in order to investigate the extraction kinetics. For extract clean-up and retention of freely dissolved target species, 30 mg of restricted-access like copolymer were used as in-line sorptive material followed by elution with methanol and on-line heart-cut injection towards a C_{18} silica reversed-phase monolithic column (100 × 4.6 mm) in a liquid chromatographic system. A mathematical model emphasized that the readily available pools *vs* time can be in most instances described by a first-order exponential equation, thus an asymptotical value is approached. Consequently, the leaching assays can be performed without attaining chemical equilibrium. Enhancement factors and detection limits were 10.2 and 18.8, and 0.40 and 0.37 mg kg^{-1} for ametryn and atrazine, respectively. The automatic method features good repeatability for leaching tests (r.s.d.: 11.8 – 10.2% for sandy and 3.7–6.2% for clayey soil). Reliable data, demonstrated with relative recoveries in the soil leachates ranging from 86 to 104%, were achieved in less than 35 min, thus avoiding the need for up to 24 h as recommended by standard leaching methods.

1. Introduction

Atrazine and ametryn are triazine herbicides often applied to weeds control, especially in maize and sugarcane crops [1]. These herbicides have been used in pre- and post-emergency periods, normally at 1.0–3.25 kg a.i. ha^{-1} doses [2]. Ametryn is usually applied once a year to maize and three-times a year to sugarcane crops [3], and residues after 198 days from initial application were estimated as 0.05 mg kg^{-1} [4]. Also, 0.08 ± 0.02 μg g^{-1} atrazine residues were determined at 100 days after applying 2.0 kg a.i. ha^{-1} of atrazine to a maize crop [5]. These data confirmed the high environmental persistence of these herbicides, which are then potential contaminant sources of river and ground-waters. Adsorption and desorption [6], photodegradation [7] and biodegradation [8] are the main processes controlling the persistence of ametryn and atrazine in soils. The adsorption and desorption processes are influenced by pH, surface area, organic matter content, particle size and porosity [9]. After desorption, the herbicides are accessible to interact with food webs. Bioaccessibility is defined as the maximal concentration of target species potentially available to biota under simulated environmental conditions and might serve as a conservative measure (*worst-case scenario*) of freely dissolved species [10–13]. There is an increasing interest in analytical partitioning methodologies to measure the fractions of bioaccessible inorganic and organic contaminants in environmental solid substrates [11,12,14,15–18]. Dilute saline solutions, such as 0.01 mol L^{-1} $CaCl_2$, 0.1 mol L^{-1} $Ca(NO_3)_2$, 1.0 mol L^{-1} NH_4OAc or 1.0 mol L^{-1} $(NH_4)_2SO_4$ have been used to correlate the bioaccessibility with the assimilation pathways of the contaminants by living organisms [11,15,17,19]. Classical batchwise procedures to access the contaminant pools of herbicides and other environmental contaminants usually use 0.01 mol L^{-1} $CaCl_2$ as extracting solution to mimic soil pore water or the percolation of rainwater through soil profiles [20–22]. These procedures however are based on end-point measurements. They do not provide information on the kinetic aspects, in spite of the fact that the involved kinetics (fast, slow or very slow) plays a fundamental role in understanding the actual hazardous effects of environmental contaminants.

For getting relevant insight into pools of bioaccessible contaminants in environmental solids, the flow analysis concept and

* Corresponding author. Tel.: +34 971172746.
E-mail address: manuel.miro@uib.es (M. Miró).

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its sequels [23] showcase advantages such as fast analysis, simple operation, minimum analyst intervention, low cost and low residues production in good agreement with the twelve principles of green chemistry [24]. The analyses are carried out in a closed environment without operator interferences, thus contaminations and/or sample losses are avoided. Flow analysis manifolds are characterized by a rigid time control and good measurement repeatability [25,26]. The sequential injection analysis (SIA) concept [27–29], an advanced modality of flow analysis, features versatile flow-programming linked to pressure-driven flow as precisely controlled by user-friendly software. The sample aliquot and reagents can be driven to other manifold compartments, such as reaction coils, solid-phase extraction (SPE) columns [28,30,31] or ancillary modules for on-line/in-line sample processing.

The goal of this work was then to propose an automatic bioaccessibility assay by harnessing an SIA analyzer for real-time monitoring of herbicide residues readily leachable from agricultural soils under simulated environmental conditions and investigation of leaching kinetics. To this end, the herbicides were extracted with a mild extractant, the extracts underwent in-line clean-up via restricted access material for removal of dissolved organic matter and colloidal species while retaining freely dissolved triazines, followed by separation with a liquid chromatograph (LC). A six-way valve with a sampling loop was accountable for both eluate heart-cut and injection of the isolated target compounds into a monolithic C_{18} column for reversed-phase LC separations. To the best of the authors' knowledge, this is the first report of automatic kinetic bioaccessibility assays for organic pollutants in environmental solids with in-line extract processing prior to fast LC separation and expedite quantification of readily mobile pools.

2. Experimental

2.1. Standards and reagents

Ultrapure water was obtained from a Milli-Q water generator (Synthesis A10, Millipore, Billerica, MA), whereas HPLC-grade methanol, acetonitrile and acetic acid were supplied by Sigma-Aldrich (Steinheim, Germany).

Atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-1,3,5-s-triazine], ametryn [2-ethylamino-4-(isopropylamino)-6-(methylthio)-1,3,5-s-triazine] and prometon [2-methoxy-4,6-bis(isopropylamino)-s-triazine], this later used as internal standard, were also obtained from Sigma-Aldrich. The stock solutions, 500 mg L^{-1} of the above-mentioned triazines, were prepared by dissolving each individual compound in pure methanol, and maintaining the solution in darkness at *ca.* 4 °C.

The extractant used in the bioaccessibility assays was a 0.01 mol L^{-1} $CaCl_2$ solution (also the carrier stream in the flow system) as per test 106 endorsed by Organisation for Economic Co-operation and Development (OECD) [21]. Working standard solutions of the target herbicides were daily prepared in this medium by stepwise dilutions of the corresponding stocks.

Non-polar styrene-divinylbenzene, copolymeric core, sorbent with hydroxylated shell (Bond Elut Plexa, Agilent, Santa Clara, CA) and hydrophilic-lipophilic balanced copolymer [poly(divinylbenzene-*co*-N-vinylpyrrolidone)] (Oasis HLB, Waters, Mildford, MA) were evaluated for in-line clean-up of leachates and concentration of triazines. Nylon syringe filters (0.45-μm pore size, Fisherbrand, Fisher Scientific, Pittsburgh, PA) were used for in-line filtration of soil leachates prior to automatic sorptive clean-up.

The SPE column (8.0 × 4.6 mm i.d) was prepared from commercially available polypropylene cartridges whereby minimal preparation was needed. The column contained about 30 mg of packed sorbent, and was connected to the flow system via an SPE tube adapter (57020-U, Sigma Aldrich) fitted to the column large-bore inlet and a barbed female luer lock fitting (Teknokroma, Barcelona) to the outlet. Polyethylene frits (10-μm pore size, Mo Bi Tec, Göttingen, Germany) were used at both ends of the column to prevent sorbent losses during system operation.

2.2. Samples

About 1.0 kg of forest soil samples were collected in agricultural areas of Piracicaba SP (Brazil) at depths of 0–20 cm. Geographic coordinates of the sampling sites were 22°37′27″S 47°36′67″W and 22°45′18″S 47°53′75″W for clayey and sandy soils, respectively.

Physicochemical characterization was accomplished by standard methods [32,33]. In brief, the samples were dried to constant weigh at 45 °C, sieved (2.0-mm mesh) and analyzed. For pH measurements, the soil suspension (1:5 w/v solid-to-liquid ratio in 0.01 mol L^{-1} $CaCl_2$) was stirred for 5 min, allowed to settle for 2 h, and stirred again prior to measurement using a combined pH electrode (Eutech Instruments, Nijkerk, The Netherlands). The pH was determined as *ca.* 3.8 for both assayed soils.

Total carbon content was determined titrimetrically as 0.8% and 2.1% for sandy and clayey soils, respectively. Regarding texture analysis using the Bouyoucos hydrometer method [32], the sandy soil was 92% sand (0.05–2.0 mm), 2% silt (2–50 μm) and 6% clay (< 2 μm), whereas the clayey soil was 26% sand, 9% silt and 65% clay.

For validation purposes, the soil samples were doped with ametryn and atrazine at the 5.0 mg a.i. kg^{-1} level [2]. To this end, 500 μL of both triazine stock solutions were added to a 25-mL volumetric flask; thereafter 12.5 mg sodium azide previously solubilized in methanol were added, and the volume was completed with methanol. The role of sodium azide is to avoid biodegradation of the triazines during the time course analysis and, hence, prevent underestimation of the concentration of potentially leachable species. The solution was dropwise added to accurately-weighed 50 g of soil until the soil particles were completely covered; homogenization was ensured by gently mixing the soil with a glass rod. The doped soils were air-dried at room temperature in darkness and aged for three weeks for stabilization.

For the bioaccessibility assays, 2.0-g of raw or doped soils were magnetically stirred with 50.0 mL of 0.01 mol L^{-1} $CaCl_2$ extracting solution, thus the 1:25 soil: extractant ratio recommended by OECD 106 [21] was maintained. A cylindrical magnetic stirrer (1.0-cm long, 2.0-mm i.d.) was used for homogenization purposes.

2.3. Apparatus

A μSIA flow analyzer (FIALab Instruments, Bellevue, WA) equipped with a 3000-step syringe pump (Cavro, Sunnyvale, CA) and a 5.0 mL gas-tight glass syringe was used for solution propelling and aspiration. The syringe was connected to an eight-port multi-position selection valve (V_2) accountable for handling the solutions involved in the batchwise soil extraction and in the in-line SPE procedure. The connection between the syringe pump and the selection valve was accomplished with a 5.0 mL holding coil made from 1.5 mm i.d. PTFE tubing. The remaining manifold tubing was of 0.8 mm i.d. The soil extracts were in-line aspirated at preset time intervals though a 0.45-μm pore size nylon syringe filter (Fisher Scientific) fixed in a PTFE tube connected to V_2. The flow system comprised two additional valves: the V_1 three-way valve, accountable for connection with the flow system or filling the syringe pump with carrier; and the V_3 six-port valve, for injection of the processed extract into LC. The system was designed to permit in a fully automatic mode the accommodation of the entire analytical method encompassing: (i) sampling of extract