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Comparison between MEKC and UV spectral deconvolution to follow sorption experiment in soil

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ABSTRACT

In this study a simple and rapid procedure for monitoring sorption of atrazine onto soil samples was developed. This method is based on a multiwavelength UV spectral deconvolution (UVSD) where the UV spectrum of a sample is considered as a linear combination of absorption spectra, named reference spectra. The combination of the reference spectra allows the restitution of the shape of the UV spectrum of any unknown sample. In order to evaluate the accuracy of this method, a sorption study of atrazine to three different soil samples was monitored using both UVSD and micellar electrokinetic chromatography (MEKC) methods. The Freundlich parameters (K_F and N) obtained for all soil samples tested were similar for both methods and UVSD has proven to be an accurate methodology, since the results did not present statistical significant differences at 95% confidence level. Moreover, the LOD obtained using UVSD presented lower values (0.066–0.12 mg L^{-1}) than the one obtained using MEKC (0.26 mg L^{-1}).

The proposed UVSD method has been proven useful as a valid alternative, to the more common procedures to follow sorption experiment in soil matrix samples with no need of sample pre-treatment or column conditioning. This method is much faster and requires less sample manipulation than traditional analytical separation methods. Moreover, most modern spreadsheet-applications, such as Excel, now include statistical packages that allow performing multi-linear regression, which make this approach particularly inexpensive since the only requirement is a UV spectrometer. The regression output was made using the Microsoft Excel, a very easy and fast program, allowing anyone, with less knowledge about complex statistical programs, to perform the UV spectral.

1. Introduction

Atrazine (2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine) is one of the most important pollutants in ground water of many countries [1,2]. Is a widely used weak-base herbicide, with a pK_a of 1.7, to control annual grasses and broadleaf weeds [3,4]. Because of its widespread use, relatively high chemical and biological stability in soils and aquifers, and high leaching potential, atrazine has been detected in surface and in ground water at high concentration levels [1,2].

The fate of organic pollutants in the environment involve complex phenomena influenced by many processes like sorption to soil components, uptake by plants, transport via runoff and leaching, biodegradation, photodegradation, volatilization, and chemical degradation [5,6]. From all of the many processes likely to occur, sorption phenomena is one of the most relevant, controlling the mobility, persistence and degradation of pollutants, such as atrazine [7–9]. For example, sorption of the pesticide to the soil will determine whether the pesticide will persist or be transported and become a pollutant (especially to groundwater) [10]. Since the behaviour of herbicides in soils greatly depends on these phenomena, a fundamental understanding of these mechanisms is critical for accurate predictions of their geochemical mobility and potential runoff to natural groundwaters [9,11].

The most used method for direct measurement of the adsorption coefficient of an organic molecule, such as atrazine, in soil is batch experiments. The main advantage of this method is the possibility to separate the soil and solution, obtaining a large volume of solution for analysis. Also the method can be easily used for routine laboratory following the OECD guideline 106 [12].

HPLC is an official method for monitoring sorption–desorption of atrazine onto soils [13–16]. This method require a long sample preparation due to clean-up procedure required before analysis by an HPLC–UV to remove interferences, such as organic matter from solution. The need of a clean-up procedure before HPLC analysis becomes clear in the work of Nemeth-Konda et al. [7] and Gao et al. [8] that used solid phase extraction before the analysis by HPLC. Several other studies have been made to follow

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the sorption–desorption phenomena using different analysis technique such as liquid scintillation [4,17–19], micellar electrokinetic chromatography (MEKC) [20] and voltammetry [11]. However, these methods can be costly and time consuming and the need of a faster, easier and less expensive technique, to follow sorption phenomena, seems to be a necessity. Atrazine absorbs in the UV region, which make UV spectrophotometry an attractive method for quantification. However, when atrazine is present in a complex matrix which also absorb in the same region creates the need to use the UV spectra deconvolution (UVSD).

UVSD is a multiwavelength deterministic or semi-deterministic procedure with an UV spectrum exploitation based on the fact that the UV spectrum of a sample is a linear combination of pre-selected representative absorption spectra called reference spectra and that the combination of the reference spectra pre-defined allows the restitution of the shape of the UV spectrum of any unknown sample [21–23]. This approach was first developed by Thomas et al. [24,25] for estimation of several wastewater parameters. Coulomb et al. [23] and Escalas et al. [26] used UVSD to determined total and dissolved organic carbon (TOC, DOC) in water samples. Moreover, Hassouna et al. [22] used this approach for quantification of water extractable organic carbon and nitrate concentrations in soil water extracts. Nam et al. [21] also used this method to quantify nitrate but this time in vegetables. Because of the growing concern about water pollution and the need of a fast, easy and low cost determination procedure, recently the UVSD was applied to monitor and detect endocrine disrupting chemicals in natural water [27].

The aim of this work was to apply the UVSD to monitor the adsorption of atrazine to three different soil samples and compare the results with the ones obtained with the MEKC method already optimized.

2. Materials and methods

2.1. Apparatus and reagents

MEKC analyses were performed using a Beckman P/ACE MDQ capillary electrophoresis system equipped with a diode array detector. Separation was carried out on an uncoated fused silica capillary 60 cm total length (50 cm effective length to the detector), 75 μm internal diameter and 375 μm of external diameter.

The UV measurements were performed using UV–vis Shimadzu spectrophotometer.

All reagents used were of analytical grade and all working solutions were prepared in ultra-pure water, obtained from a Milli-Q Millipore (Millipore Q plus 185) system. A stock standard solution of atrazine (97.4%, Riedel-de Haën) was prepared in methanol and standard solutions were prepared by diluting this stock solution with 0.01 M $CaCl_2$. A stock of internal standard (IS) solution, ethylvanillin (99%, Aldrich) was prepared, dissolving in a small quantity of acetonitrile and completing with ultra-pure water. Run buffer used was prepared weekly with 10 mM of $NaH_2PO_4{\cdot}2H_2O$ (99%, Fluka) and 50 mM sodium dodecyl sulphate (SDS) (99%, Panreac) in ultra-pure water. Buffer pH was adjusted to 8.50 ± 0.02 with 1 M NaOH.

2.2. Soil sample

The soil sample was collected (0–30 cm) from a long-term field experiment which was established in 1962, at the experimental farm of INRES - Institute of Plant Nutrition, University of Bonn, on a luvisol derived from loess (17.8% clay, 67.3% silt, 5.9% sand), following a cereal-root crop sequence [28]. The soils samples used were subjected to different fertilizations: sewage sludge from municipal wastewater treatment facilities (14.88 t ha^{-1}) (SLU), farmyard manure (9 t ha^{-1}) (FYM) and mineral fertilizer (MIN). The organic carbon content was 1.98%, 1.47% and 1.24, respectively. Soil samples were air-dried and passed through a sieve, mesh size 2 mm.

2.3. Adsorption experiment

Adsorption isotherm of atrazine was made using the batch equilibration technique [12]. Five pesticide concentrations (2–10 mg L^{-1}) were prepared in 0.01 M calcium chloride. A 4 mL aliquot of each concentration of atrazine solution was added to 2 g of soil. Five adsorptions were made for each concentration. The tubes containing the mixtures were shaken, head over head at 100 rpm for 24 h at 20 ± 1 °C, centrifuged and the supernatant filtered and analysed. The equilibrium concentration after the sorption experiment was determined using MEKC and UVSD.

2.4. MEKC analysis

After adsorption experiment, samples were filtered through a 0.22 μm filter (Millex-GV from Millipore), internal standard was added and analysed directly by MECK as described previously [20]. Standards, with concentrations ranging from 0.3 and 10 mg L^{-1}, and samples were injected using 0.5 psi pressure during 3 s. Electrophoretic separations were carried out at positive power supply of 20 kV for 7 min, maintaining the capillary temperature at 25 °C, resulting in a current of ~60 μA. Atrazine and ethylvanillin were monitored by detection at 214 nm or in the range 190–600 nm for multiwavelength detecting. The run buffer vials were used for six consecutive injections before the replacement with new vials.

2.5. UV determination

Standards, with concentrations ranging from 0.1 and 10 mg L^{-1}, for UV determination were prepared in the raw soil water extract containing 0.01 M $CaCl_2$. UV absorbance reference spectra (REF_1, REF_2, REF_3) were registered for the raw soil water extract, for the 0.01 M calcium chloride and for an atrazine solution in 0.01 M $CaCl_2$. Ammonium hydroxide was added to standards, samples and reference solutions in order to obtained 0.1 M as final concentration and all of them were diluted five times prior to analysis, resulting in a calibration curve from 0.1 to 2 mg L^{-1}.All samples were diluted 5 times to avoid UV spectra saturation obtained prior to the dilution. Ammonium hydroxide was used to buffer the solution and improve the repeatability and to avoid the formation of aggregates of organic matter. However, as a consequence of increasing the pH of the solutions, a calcium hydroxide precipitate was observed and removed by centrifugation and filtration of the samples and standards.

The UV absorbance was performed using 1 cm light-path UV micro cuvettes Plastibrand from Brand and was registered at 1 nm bandwidth between 220 and 300 nm.

2.6. Principle of UVSD

The principle of application of the UVSD approach, subject of some publications [21–28], is a multiwavelength approach based on a procedure of matrix algebra where each spectrum corresponds to a linear combination of a small number p of reference spectra. In the model, the coefficients a_i of the linear combination are calculated by the resolution of a system based on the following relation established for each wavelength:$S_s = \sum_{i=1}^{p} a_i \mathrm{REF}_i(\lambda) \pm rS_s$ is the sample absorbance, REF_i (λ) is the absorbance of the ith REF at each wavelength λ, a_i the contribution coefficient of the ith REF in the linear combination, p the number of reference spectra and r

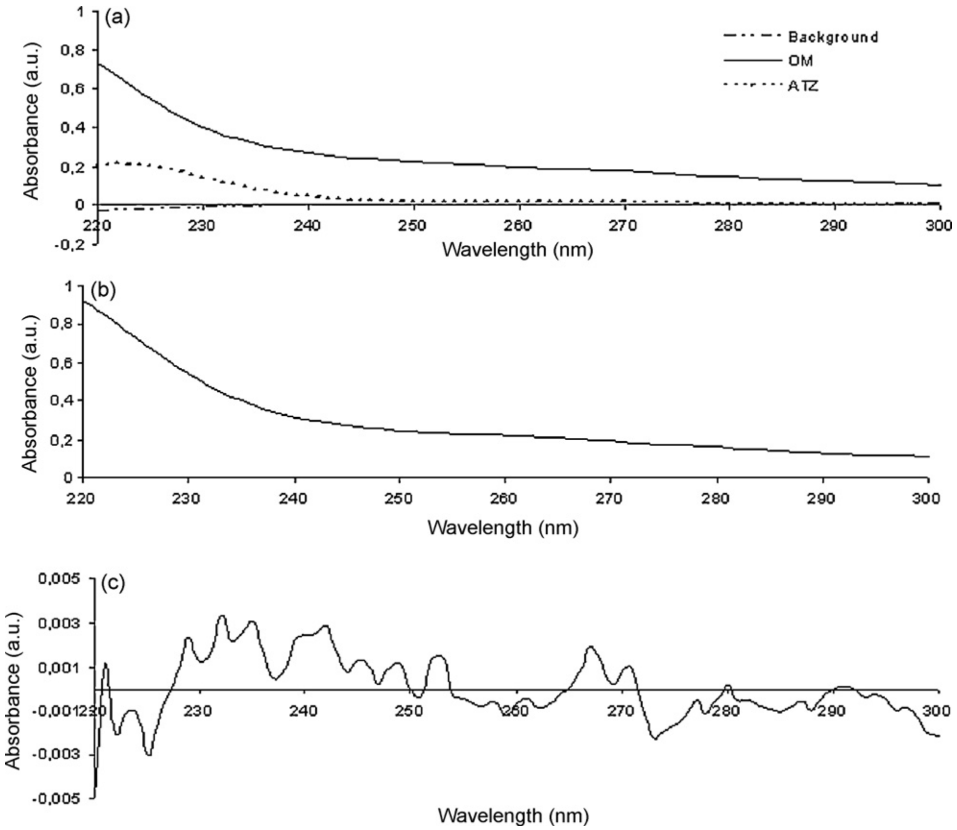

Fig. 1. UV spectra from adsorption experiments on soil fertilized with sewage sludge: (a) UV Reference spectra of background (0.01 M $CaCl_2$), dissolved organic matter and atrazine; (b) combined spectrum of the three reference spectra; (c) residual plots.

is the error of restitution at each λ [21–23]. The associated contribution coefficients (a_i) of each reference spectra in the linear combination are calculated using several complexes programs. In this paper we performed these calculations using the simple common program denominated Excel. The regression output using the Microsoft Excel is very easy and fast, allowing anyone, with less knowledge about complex statistical programs, to perform the UV spectral deconvolution and to obtain the associated contribution coefficients (a_i) of each reference spectra, the correspondent standard deviation, the standard deviation of the calculation and also the deconvolution residuals.

2.7. Sorption data treatment

The Freundlich parameters (K_F and N) were calculated from the fitting, of non-linear regression of the equation $Q_e = K_F \times C_e^N$, to the experimental data, where Q_e is the total sorbed concentration (mg kg^{-1}), C_e is the solution-phase concentration (mg L^{-1}), K_F (mg kg^{-1})(mg L^{-1})$^{-N}$ is the Freundlich distribution coefficient, and N is the isotherm nonlinearity factor. Isotherms were plotted (Q_e vs. C_e), and K_F and N were obtained after performing the non-linear regression.

3. Results and discussion

3.1. UVSD method

Also all samples measured, using UV–vis spectrophotometer, were subjected to centrifugation and filtration prior to the analysis. Preliminary studies showed the importance of these two steps in reproducibility of the obtained spectra, since the precipitation formed, due to the addition of the ammonium hydroxide as buffer solution, interfere in the UV spectrum of each sample. An example of the three reference spectra, background, dissolved organic matter and atrazine spectra obtained for soil fertilized with sewage sludge, composing the deconvolution basis are represented in Fig. 1a. The combined spectrum of the three reference spectra is represented in Fig. 1b. The residuals plot is showed in Fig. 1c and is the difference between the observed value and the corresponding value given by the regression linear function at each wavelength.

The plot of the residuals is very helpful to detect an existence of an obvious correlation between the residuals and the independent variable x, and to evaluate if the chosen model is adequate or not to fit the experiment. The fact that the residuals look random (Fig. 1c) is an indication that there is no obvious correlation with the variable x.

3.2. Analytical curves by UVSD and MEKC

The analytical experimental procedure used in the present work is based on a previous work where an optimization of a MEKC procedure to follow sorption experiments of atrazine onto a soil sample [20].

The UVSD method was performed using standards by spiking atrazine into the same soil matrix as the one present in the adsorption experiments. The calibration curve was obtained plotting the coefficient a_i of the linear combination, obtained for each atrazine standard using the linear regression, in function of the atrazine concentration. Since spectrum depends on soil matrix, a calibration curve is needed for each soil sample.

Table 1 shows statistical parameters of the analytical curves obtained either by UVSD and MEKC used for the determination of the concentration of atrazine present in solution after the adsorption experiment.

Table 1
Statistical parameter (± standard errors) for typical analytical curves obtained by MEKC and by UV spectral deconvolution for each soil sample tested.

	Slope	Intercept	R^2	LOD ($mg\,L^{-1}$)	Linearity
UVSD (SLU)[a]	0.83 ± 0.01	−0.014 ± 0.007	0.9966	0.066	99.986
UVSD (MIN)[a]	0.85 ± 0.02	0.005 ± 0.008	0.9957	0.074	99.984
UVSD (FYM)[a]	0.91 ± 0.01	−0.05 ± 0.02	0.9982	0.12	99.986
MEKC[b]	2.75 ± 0.02	−0.03 ± 0.02	0.9994	0.26	99.983

[a] Statistical parameters valid for the concentration range between 0.1 and 2 $mg\,L^{-1}$.
[b] Statistical parameters valid for the concentration range between 0.3 and 10 $mg\,L^{-1}$.

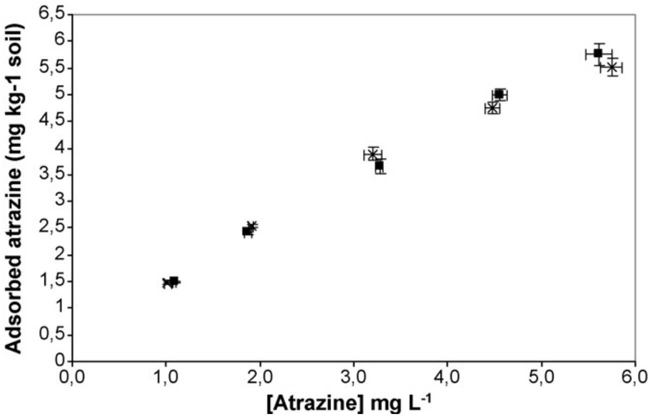

Fig. 2. Adsorption isotherms of atrazine onto soil fertilized with sewage sludge obtained by UVSD (■) and by MEKC (×).

Comparing the results we can observe that both methods present a good correlation coefficient, very close to 1. In terms of LOD, the UVSD presents a much lower value 0.066 and 0.12 $mg\,L^{-1}$ than the one obtained using MEKC 0.26 $mg\,L^{-1}$. In what concerns the linearity, the values are very similar to each other.

3.3. *Adsorption experiments—evaluation of the analytical methods*

Adsorption isotherm were obtained by plotting the amount of atrazine sorbed per unit weight of soil at equilibrium (Q_e, $mg\,kg^{-1}$) versus the amount of chemical per volume of solution at equilibrium (C_e, $mg\,L^{-1}$). The adsorption isotherms of atrazine onto soil fertilized with sewage sludge obtained by the proposed UVSD method in comparison to the curve obtained by MEKC are presented in Fig. 2.

The Freundlich equation has reasonably described the adsorption of atrazine on soils used ($r^2 > 0.927$). Freundlich adsorption coefficient is an empirical constant of the Freundlich model expressing soil sorbent capacity (sorption isotherm slope) for a given range of atrazine concentration and greater the value, stronger is the adsorption. The low K_F value obtained for mineral soil (Table 2), using both methods, reflects low adsorption capacity and is commonly associated with greater permeability and high leaching [2]. Sorption isotherm was non-linear, exhibiting N values smaller than 1, indicating that the percentage of atrazine adsorbed to the soil decreased as the initial concentration increased.

Table 2
Mean (± standard errors) Freundlich K_F and N parameters for adsorption of atrazine onto a soil obtained by UVSD and MEKC.

Soil sample	K_F ($mg\,kg^{-1}\,(mg\,L^{-1})^{-N}$)	N	R^2
SLU			
UVSD	1.45 ± 0.07[a]	0.8 ± 0.3	0.987
MEKC	1.54 ± 0.06	0.76 ± 0.03	0.989
t student[b]	1.72	0.201	
MIN			
UVSD	0.68 ± 0.1[a]	0.85 ± 0.09	0.927
MEKC	0.81 ± 0.08	0.72 ± 0.05	0.991
t student[b]	1.77	2.21	
FYM			
UVSD	1.9 ± 0.1[a]	0.61 ± 0.04	0.992
MEKC	1.7 ± 0.2	0.67 ± 0.08	0.974
t student[b]	1.39	1.23	

[a] Results are an average of triplicate of adsorption experiments.
[b] Critical *t* value for 4 degrees of freedom at 95% confidence level is 2.78.

The *t*-test was applied to compare the results obtained by both methods for each soil sample used. Since the *t* calculated presented lower values for both K_F and N in all soil samples tested than the critical *t* value for 4 degrees of freedom at 95% confidence level, it is possible to confirm that there are no significantly differences between the results obtained by UVSD and MEKC.

4. Conclusion

In the present work we demonstrate that the monitoring of adsorption of atrazine onto different soil samples can be correctly determined using mathematical deconvolution of UV-spectra. This was proved by the determination of the Freundlich parameters (K_F and N) obtained for the adsorption isotherm, using UVSD and MEKC as analyses techniques. UVSD has proven to be an accurate methodology, since the results obtained did not present statistical significant differences at 95% confidence level. Moreover the LOD obtained using UVSD presented a much lower value than the one obtained using MEKC.

This is particularly interesting for the study of the interactions of pesticides to soil, a field of growing importance. Indeed whereas in batch experiment high parallelism is possible, the measure of the compound remaining in solution is often the bottle-neck of the approach, where the concentration in each batch has to be measure sequentially. When using HPLC or CE, each measurement, including pre-treatment, column equilibration, and separation, will take between 15 and 60 min in average depending of the compound, without considering the method development. Here a recording take less than 1 min. It is important to emphasize that this approach is reliable only if the spectra of the matrix and the compound used as reference are invariant.

This approach should be applied to study the adsorption of several other compounds to different soil samples, as long as the compounds is stable enough for its spectra to be consider invariant for a relatively short period of time. The cost of this approach is also particularly low as the only requirements are a UV–vis spectrometer and a spreadsheet with statistical tool (i.e. Excel).

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