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Development of an ELISA procedure to study sorption of atrazine onto a sewage sludge-amended luvisol soil

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ABSTRACT

Pesticides may contaminate ground and surface waters and one of the major factors governing this property is soil sorption. Sorption can be assessed by batch equilibrium technique which produces lots of extracts with high dissolved organic carbon concentration in which the pesticide concentration has to be determined. We developed an ELISA procedure to analyse atrazine based on polyclonal antibodies (C193) for which tracer structure and dilutions of immunochemical reagents were adapted to fit the purpose. After a 1000-fold dilution (or after an SPE clean-up procedure) extracts of a sewage-sludge amended luvisol (used as an example application of the methodology developed) could be reliably analysed. The Freundlich model is able to describe adsorption for this system ($r^2 = 0.977$) delivering a distribution coefficient K_F of 1.6 ± 0.2 $(\text{mg kg}^{-1})(\text{mg L}^{-1})^{-N}$ and an isotherm nonlinearity factor N of 0.70 ± 0.09.

1. Introduction

Pesticides are important in modern farming, being practically impossible to produce the enormous quantities of food that are required to feed the world's growing population without their use. However, pesticides may constitute also a significant source of contamination of air, water, soil and food products [1]. Some are stable in the environment, undergo bioconcentration along the trophic chains and can be toxic to insects, fish and other water organisms, birds and mammals, including humans. The bioconcentration of these compounds is therefore considered to be hazardous for organisms on the top of the trophic pyramid [2].

Among the herbicides, some of the most frequently used belong to the triazines group [2]. The most prominent member of this group is atrazine (2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine) a weak-base, with a pK_a of 1.7 [3,4], moderately soluble in water (28 mg L^{-1} [5]) and widely used to control annual grasses and broadleaf weeds. Atrazine constitutes one of the most important pollutants in ground water of many countries [6,7] and it is therefore important to monitor its concentration in the environment [2].

Atrazine has been recently recognized as an endocrine disruptor for mammals and aquatic life [8]. Because of the ubiquitous and inevitable water contamination, its use has been banned in the European Union countries in 2003, legislation permitting only a very low concentration of atrazine in drinking water (0.1 μg L^{-1}) [8,9]. As a consequence of its solubility, high chemical and biological stability in soils and aquifers, widespread use and considerable leaching potential, atrazine has been detected in surface, ground, and drinking waters, sometimes at elevated concentrations exceeding the permissible levels [6,7,9,10].

The fate of organic pollutants in the environment involves complex phenomena [11,12] and from all of the many processes likely to occur, sorption phenomena are of the most relevant, controlling mobility, persistence and degradation of pollutants such as atrazine [13–15]. As a result, the sorption determines either the pesticide will persist in the top soil layer or be transported and become a pollutant (especially to groundwater) [16,17].

The adsorption coefficient of an organic molecule, such as atrazine, in soil, is usually assessed via batch equilibrium experiments following the OECD guideline 106 [18]. This method is easily used for laboratory routine with the main advantage of separating the soil and solution obtaining a large volume for analysis. Sample preparation for traditional analytical detection techniques, can involve considerable time in extracting and concentrating pesticide residues before analysis [19]. HPLC is the most commonly used method for monitoring sorption of atrazine onto soils [20–23] but also requires a long sample preparation due to clean-up procedures necessary prior to analysis. Other possible techniques to follow sorption phenomena are liquid scintillation counting (LSC) [4,24–26], micellar electrokinetic chromatography (MEKC) [27] and voltammetry [17], however these methods can be costly and time consuming. Conversely, an enzyme-linked immunosorbent assay (ELISA) offers a way to quantitatively determine residual pes-

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ticide concentrations in water and soil samples with much less effort, higher sensitivity, simplicity, quicker sample turnover time and often no need for sample clean-up or pre-concentration steps [19,28]. Accordingly, ELISA has been favourably compared with GC analysis when used for the evaluation of pesticide levels in surface and groundwater samples [29–31].

However, like in all analytical procedures, when using enzyme immunoassays on soil extracts some problems may arise due to co-extracted cross-reacting compounds or matrix effects. Matrix effects may produce false-positive results derived from the interference due to synthetic or natural substances, including structurally similar compounds, halogens and dissolved organic carbon (DOC), that interact with the antibody or the enzyme tracer impairing binding or activity of the tracer, leading to a lower signal [19,32]. The effect of DOC on standard solutions in an immunoassay has been assessed before and matrix effects were attributed to interference with the attachment of atrazine to the antibody [19]. However, diluting the soil extract in water, the possible matrix effects were reduced [33].

The aim of this study was to develop an ELISA technique that could be used in the determination of atrazine in soil sample batch equilibrium solutions in order to follow the sorption behaviour of this pesticide onto soil samples. The optimized ELISA and a previously developed MEKC methodology [27] were applied to a soil sample and results compared.

2. Materials and methods

2.1. 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 [34]. The soil was amended with sewage sludge from municipal wastewater treatment facilities (14.88 t ha^{-1}). Therefore the organic carbon content of the selected soil was relatively high (1.98%) and soil pH was 6.5. The soil sample was air-dried and passed through a sieve, mesh size 2 mm.

2.2. ELISA

2.2.1. Reagents and materials

All reagents were of analytical or biochemical grade and were used as received. Horseradish peroxidase (HRP) (EIA grade) was obtained from Roche, Guardian™ (an enzyme stabilizer) was from Perbio. Tetramethylbenzidine (TMB) and Tween™ 20 were purchased from Serva, sodium azide was from VWR, ultrapure water was obtained by running demineralized water through a Milli-Q water purification system from Millipore. Atrazine and all buffer salts were supplied by Sigma–Aldrich. Sephadex columns were from Amersham Biosciences. For the immunoassay the following buffers were used: washing buffer concentrate (60×) (43 mmol L^{-1} KH_2PO_4, 375 mmol L^{-1} K_2HPO_4, 1.33 mmol L^{-1} sorbic acid potassium salt and 3% Tween™ 20, pH 7.6), PBS buffer (10 mmol L^{-1} $NaH_2PO_4{\cdot}2H_2O$, 70 mmol L^{-1} $Na_2HPO_4{\cdot}2H_2O$, 145 mmol L^{-1} NaCl, pH 7.6), coating buffer (15 mmol L^{-1} Na_2CO_3, 35 mmol L^{-1} $NaHCO_3$, 3 mmol L^{-1} NaN_2, pH 9.6) and substrate buffer (200 mmol L^{-1} citric acid monopotassium salt buffer containing 3 mmol L^{-1} H_2O_2 and 0.01% sorbic acid potassium salt, pH 3.8). A stabilized TMB solution (41 mmol L^{-1} TMB, 8 mmol L^{-1} tetrabutylammoniumborohydride in dimethylacetamide) was prepared according to Frey et al. [35]. The final substrate solution consists of 540 μL TMB solution in 21.5 mL substrate buffer and was freshly prepared for each run. Microtiter plates used were transparent with 96 flat-bottom wells possessing high binding capacity (MaxiSorp™, Nunc). Washing steps were carried out using an automatic plate washer (Atlantis, Asys Hitech). The incubation of the plates was performed using a plate shaker (Titramax 100, Heidolph). Absorbance was measured at 450 nm and referenced to 620 nm with a plate reader (UVM 340, Asys Hitech).

2.2.2. Antibody

Antiserum production for the polyclonal antibody C193 was described by Wittmann and Hock [36]. Direct ELISA procedure and suitability of this antibody for sensitive atrazine analysis has been reported [37–39].

2.2.3. Preparation of enzyme conjugates

Six triazine derivatives have been selected for tracer synthesis (Fig. 1).

Syntheses and naming of derivatives were carried out by Weller [40,41], as well as initial comparison of their binding properties [40,42].

In order to produce enzyme tracers of different structure the six selected triazine derivatives were coupled to HRP using modifications of the active ester method described by Schneider and Hammock [43]. Triazine derivatives (10 μmol), 20 μmol of N-hydroxysuccinimide (NHS) and 10 μmol N,N-dicyclohexylcarbodiimide (DCC) were dissolved in 120 μL tetrahydrofuran (THF) and stirred at room temperature overnight. The mixture was centrifuged at 1750 × g for 10 min, 6 μL of the THF supernatant was added dropwise to 200 μL stirred peroxidase solution (2.21 mg HRP in 1 mL 0.13 mol L^{-1} $NaHCO_3$). Stirring at room temperature and protecting the solution from light was continued for 5 h. For purification, PBS buffer diluted 10 times was used for elution of the enzyme conjugates from Sephadex G-25 PD10 columns, fractions were collected in a microtiter plate and their optical density (OD) measured at 405 nm. Fractions of highest OD values were pooled, mixed with an equal amount of Guardian™, aliquoted and stored at 4 °C.

2.2.4. ELISA procedure

Direct competitive ELISA was adapted for the analysis of atrazine. Microtiter plates were coated with polyclonal antibody serum diluted 1:20,000 in coating buffer using 200 μL per well. Plates were covered with Parafilm™ to prevent evaporation. After overnight incubation at 20 °C in the plate shaker at 750 rpm, the plates were washed three times with wash buffer concentrate diluted 60 times. After the three washing cycles standards/samples were added to the plate (100 μL per well) and the plate shaken at room temperature for 30 min. This was followed by addition of the respective enzyme conjugate in PBS (diluted 1:10,000, 100 μL per well); the plate was shaken at room temperature for 30 min, followed by a second three-cycle washing step. Finally, the substrate solution was added (200 μL per well) and incubated for 30 min. The enzyme reaction was stopped by addition of sulphuric acid 1 mol L^{-1} (100 μL per well).

Absorbance was read at 450 nm and referenced to 620 nm. All determinations were at least made in triplicate. The mean values were fitted to a four-parametric logistic equation (4PL) [44]. Data comparison between assay variations was performed by normalizing the absorbance using the following formula:

$$\text{Normalized}\quad \text{OD} = \frac{\text{OD}\quad \text{std} - D}{A - D} \times 100$$

where OD std: absorbance at respective standard concentration, A: absorbance at zero concentration, and D: absorbance at excess standard concentration [45].

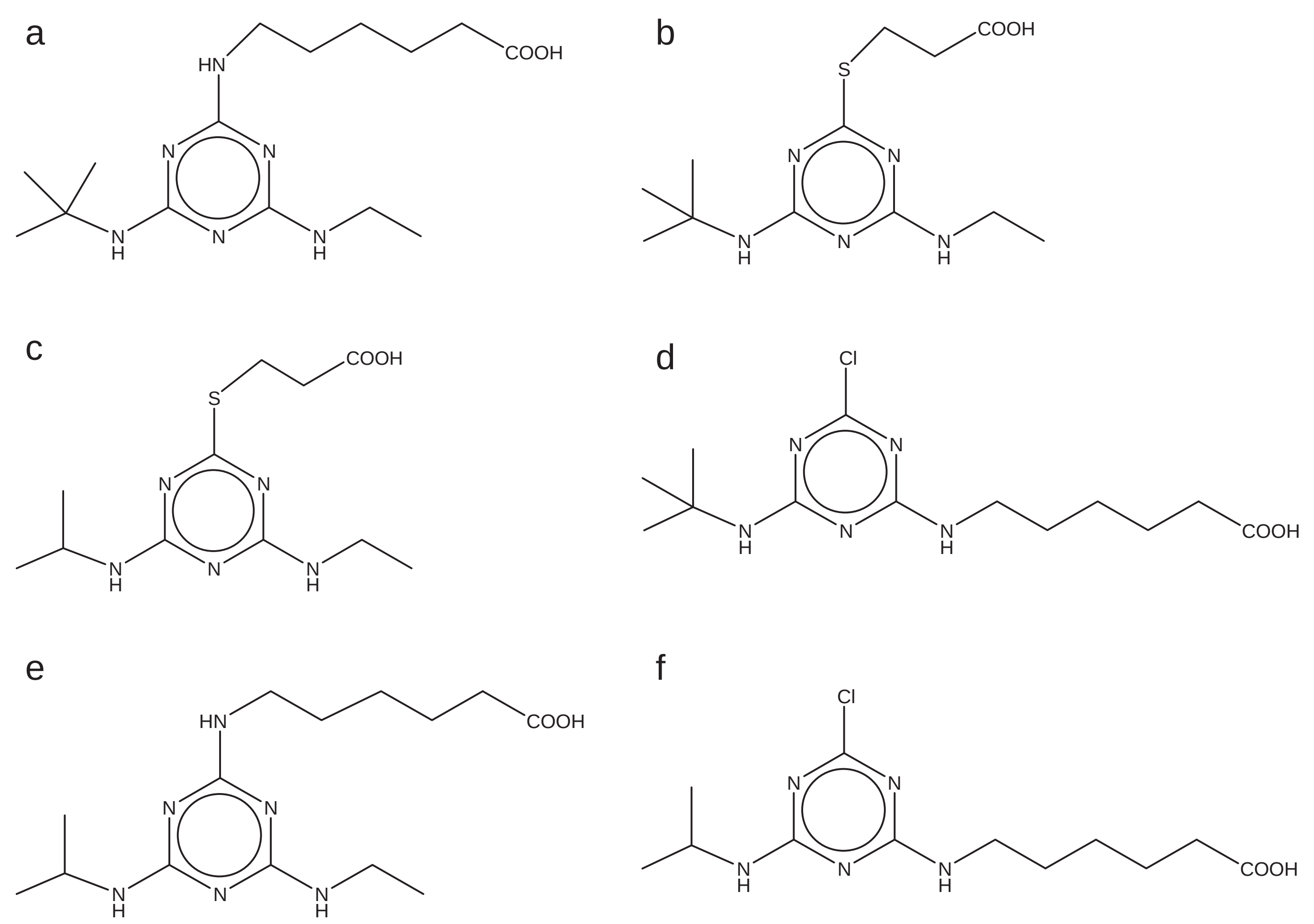

Fig. 1. Triazine derivatives: (a) 2-(tert-butylamino)-4-[(1-carboxypent-5-yl)amino]-6-(ethylamino)-1,3,5-triazine (t-Bu/Et/C6); (b) 2-(tert-butylamino)-4-[(1-carboxyeth-2-yl-)thio]-6-(ethylamino)-1,3,5-triazine (t-Bu/Et/SC3); (c) 2-[(1-carboxyeth-2-yl-)thio]-4-(ethylamino)-6-(isopropylamino)-1,3,5-triazine (i-Pr/Et/SC3); (d) 2-(tert-butylamino)-4-[(1-carboxypent-5-yl)amino]-6-chloro-1,3,5-triazine (t-Bu/Cl/C6); (e) 2-[(1-carboxypent-5-yl)amino]-4-(ethylamino)-6-(isopropylamino)-1,3,5-triazine (i-Pr/Et/C6); (f) 2-[(1-carboxypent-5-yl)amino]-4-chloro-6-(isopropylamino)-1,3,5-triazine (i-Pr/Cl/C6) (for nomenclature, cf. to [40]).

2.2.5. Tracer binding specificity

In order to evaluate and choose the most appropriate synthesized tracer for the polyclonal antibody C193, the OD for two standards of atrazine (blank and 1000 $\mu g\,L^{-1}$) were determined using the 6 different tracers. All determinations were made in triplicate. The evaluation was made by comparison of the OD obtained for each standard in order to assess tracer binding (high blank signal), important for signal generation, against analyte competition (lowering OD) which determines sensitivity of the resulting assay.

2.3. Adsorption experiment

Adsorption isotherms of atrazine were established using the batch equilibration experiments [18] in a miniaturized approach analogously to Fruhstorfer et al. [46]. Five pesticide concentrations (2–10 $mg\,L^{-1}$) were prepared in 0.01 $mol\,L^{-1}$ calcium chloride solution. A 4 mL aliquot of each concentration of atrazine solution was added to 2 g of soil. Three adsorption trials were performed for each concentration. The tubes containing the mixtures were shaken, head over head at 100 rpm for 24 h at $20 \pm 1\,^{\circ}C$, centrifuged and the supernatant filtered and analysed. The equilibrium concentration after the sorption experiment was determined using MEKC and ELISA. For ELISA determination, sample solutions were diluted 10,000 times. MEKC analysis procedure was performed as described previously [27].

2.4. Sample clean-up procedure

In cases when a 10,000-fold sample dilution is not possible due to a low atrazine concentration present in matrix solution a solid-phase extraction (SPE) procedure was adopted. SPE cartridges, from Phenomenex Inc., STRATA X, size 6 mL were conditioned by flushing with 3 mL $CHCl_3$, followed by 6 mL of methanol, 6 mL of ultrapure water and 6 mL of 0.01 $mol\,L^{-1}$ $CaCl_2$ solution. The 1000 $\mu g\,L^{-1}$ atrazine samples (5 mL) were passed through the cartridges with a flow of ca. 1–2 $mL\,min^{-1}$, then the cartridges were rinsed with 3 mL of water. After the washing step, the cartridges were dried completely by a nitrogen stream for 1 h and the analyte was eluted with 5 mL of $CHCl_3$. The eluate was reduced to dryness under a gentle stream of nitrogen and recovered with 5 mL of ultrapure water. In order to evaluate the complete removal of sample organic matrix, sample UV spectra, before and after SPE procedure, were recorded between 300 and 220 nm, using a Shimadzu UV-1603 Spectrophotometer UV–Vis System, in a 1 cm quartz cell. In order to determine the recovery of the SPE procedure, samples (three replicates) were analysed using MEKC according to the method previously described by Lima et al. [27].

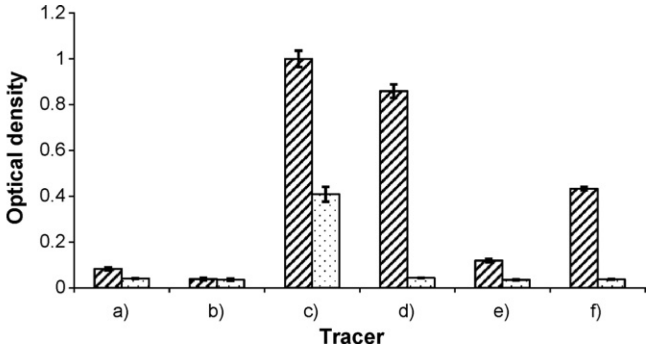

Fig. 2. OD results and error bars for $n = 3$ for blank (▨) and 1000 μg L^{-1} (⊡) atrazine standard using the tracers obtained from coupling the following triazine derivatives: (a) t-Bu/Et/C6; (b) t-Bu/Et/SC3; (c) i-Pr/Et/SC3; (d) t-Bu/Cl/C6; (e) i-Pr/Et/C6; (f) i-Pr/Cl/C6; antibody dilution 1:5000; tracer dilution 1:5000 each.

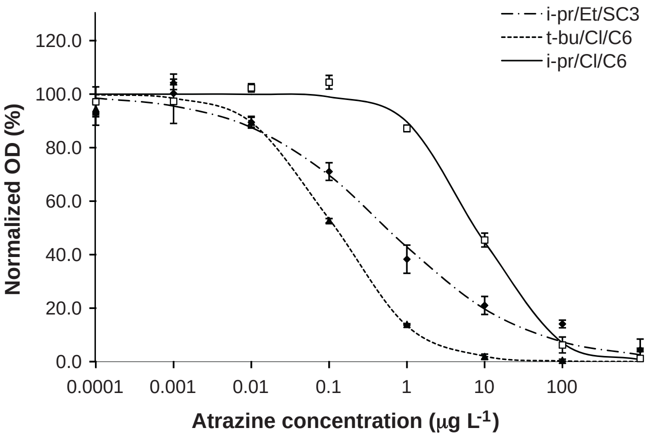

Fig. 3. Calibration curves obtained for direct ELISA using pab C193 and enzyme tracers produced for the 3 triazine derivatives given in the legend. *Y*-axis is normalized OD according to the equation given in Section 2.2.4; antibody dilution 1:5000; tracer dilution 1:5000. Equation parameters: i-Pr/Et/SC3 ($A = 0.792$; $B = 0.486$; $C = 0.520$; $D = 0.464$); t-Bu/Cl/C6 ($A = 0.798$; $B = 0.869$; $C = 0.117$; $D = 0.041$); i-Pr/Cl/C6 ($A = 0.359$; $B = 1.028$; $C = 8.127$; $D = 0.027$).

Table 1
Parameter values obtained for the four-parametric logistic equation (4PL) using different antibody and tracer dilutions.

Tracer	*A*	*B*	*C*	*D*
Ab 1:20,000 Tracer 1:20,000	0.329	0.996	0.087	0.024
Ab 1:20,000 Tracer 1:10,000	0.464	0.922	0.095	0.026
Ab 1:10,000 Tracer 1:20,000	0.358	0.911	0.084	0.021
Ab 1:10,000 Tracer 1:10,000	0.478	0.910	0.083	0.027

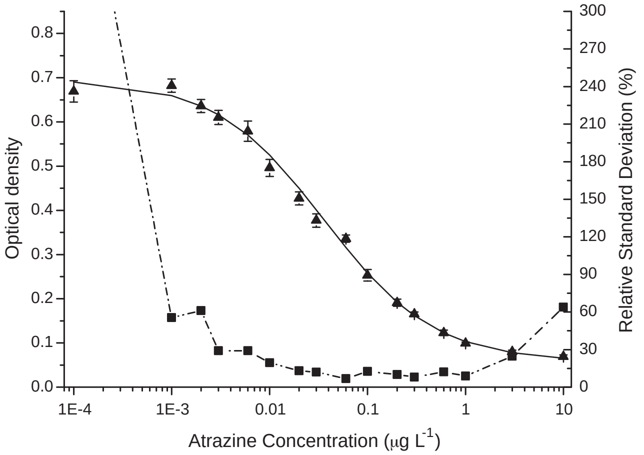

Fig. 4. Calibration curve (triangles) of ELISA ($A = 0.697$; $B = 0.776$; $C = 0.036$; $D = 0.058$; $r^2 = 0.996$) and precision profile (squares). The precision profile and determination of the relative error of concentration were calculated in accordance with Ekins [47].

3. Results and discussion

3.1. ELISA adaptation

3.1.1. Tracer binding specificity

OD results obtained for both atrazine standards assayed (blank and 1000 μg L^{-1}) with the 6 different tracers synthesized are presented in Fig. 2.

From Fig. 2, it is possible to verify that using the tracer derived from t-Bu/Et/SC3 the OD obtained for a high atrazine standard is similar to the one obtained with the blank, denoting an unspecific binding that allowed us to conclude that this tracer is not suitable for the determination of atrazine using this polyclonal antibody. Looking to the rest of the results, the OD obtained for the blank is always larger than the one obtained for the atrazine standard 1000 μg L^{-1}. However, the difference between the OD is significantly larger using i-Pr/Et/SC3, t-Bu/Cl/C6 and i-Pr/Cl/C6 tracers. For this reason, and in order to evaluate the most appropriate tracer, the complete calibration curves (Fig. 3) and the correspondent parameters of the 4-parameter-fitting (4PL) were determined for each of the three mentioned tracers. The parameters correspond to A = signal at zero dose, D = signal at excess dose, C = x-coordinate (concentration) of the turning point of the sigmoidal curve and B = slope parameter.

From the three tracers tested the t-Bu/Cl/C6 was chosen since it presents the lowest turning point value (C), indicating the possibility to achieve a lower detection limit. Also this tracer presented a higher OD difference between the blank (A) and the maximum concentration used (D).

3.1.2. Optimization of ELISA procedure

After choosing the suitable tracer for the atrazine quantification it was necessary to optimize the antibody (Ab) and tracer dilution. Four combinations were tested: Ab diluted 1:10,000 and 1:20,000 and tracer diluted 1:10,000 and 1:20,000. The parameters obtained for the four-parametric logistic equation curve fitting using the different combinations of antibody and tracer dilution are presented in Table 1.

Although the parameter values obtained were not significantly different, the combination that presented the lowest turning point (0.083) and the highest A parameter value (curve upper limit) was chosen (antibody and tracer dilution 1:10,000).

3.1.3. Evaluation of assay performance

After performing the optimization of the ELISA conditions the assay had to evaluated in terms of precision profile. Also, the concentration working range had to be defined. ELISA calibration curve and respective precision profile, determined as described by Ekins [47], are presented in Fig. 4. In order to determine the quantification range of the previous optimized assay, 16 standards with concentrations ranging from 0.0001 to 10 μg L^{-1} (six replicates per standard) were used. Assessing the quantification range via the precision profile allows to know the highest and lowest concentration which can be quantified with an acceptable degree of precision and is calculated using the respective precision profile with a maximum RSD (relative standard deviation) of the concentration result of 30%. Considering this RSD as the maximum value allowed, the analytical

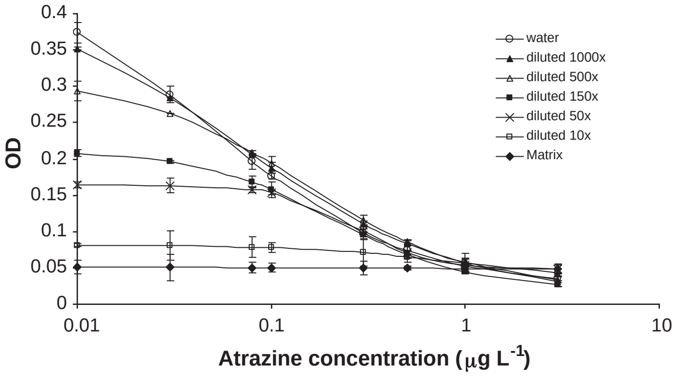

Fig. 5. Influence of soil matrix on atrazine determination by ELISA. Shown are the calibration curves obtained from standard solutions prepared in raw extract matrix of the soil (described in Section 2.1) and in subsequent matrix dilution. A calibration curve obtained from calibrators prepared in ultrapure water is also shown for comparison.

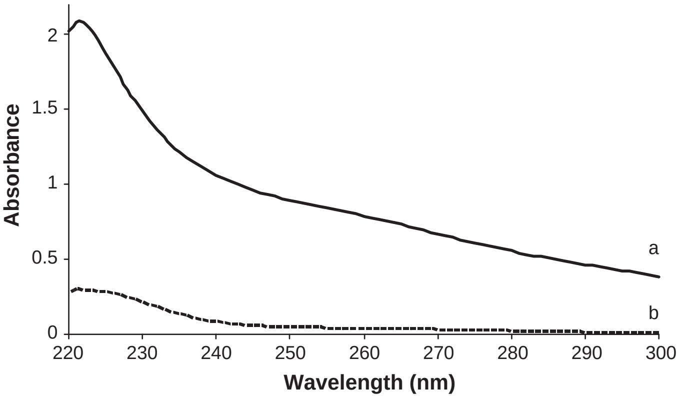

Fig. 6. UV spectra of an equilibrium extract prepared as described in Section 2.3 from a luvisol soil amended with sewage sludge before (a) and after (b) SPE clean-up.

working range for atrazine quantification obtained was between 0.003 and 3.0 $\mu g\,L^{-1}$. This assay can be used over three orders of magnitude of analyte concentration.

3.1.4. Evaluation of matrix effects on optimized ELISA procedure

Soil matrix effects on the ELISA were evaluated using calibration curves obtained with standards prepared both in soil extract and in several dilutions of it. According to Fig. 5, with increasing concentration of matrix in the standards the sigmoidal shape of the calibration curve was steadily lost, i.e., quantification turning impossible. Since organic matter present in soil solution is a complex mixture of aromatic and aliphatic hydrocarbon structures linked by and bearing many functional groups [48], the intrinsic mechanisms of interference are unknown. We suppose that it may be a combination of different effects as stated already by other researchers [49]. It is important to note from these findings that for samples from the sorption experiments carried out analysis by ELISA should be possible after dilution of a factor of 1000.

3.2. Sample clean-up procedure

Comparing the UV spectra of the soil extract before and after the SPE clean-up procedure described (Fig. 6) it is possible to observe a significant absorbance decrease in UV spectrum, especially below 230 nm. This is due to the removal of organic matrix which presents a high absorbance signal at low wavelengths, indicating the efficiency of the clean-up procedure. In order to evaluate the recovery of the applied SPE method, samples were analysed using MEKC, previously and after sample clean-up. Results indicated a recovery between 98 and 103%, making this approach a valid alternative every time dilution is not a possible way to reduce matrix interference in the ELISA determination. Also, it is important to highlight that this sample clean-up method could be useful in water samples with low atrazine level, where sample dilution can not be performed in order to reduce matrix effects. In our work, since the concentrations used to perform adsorption experiments were in a range where the dilution allowed quantification by ELISA, without any matrix interference, no SPE procedure was needed.

Table 2
Mean (±standard errors) Freundlich K_F and N parameters for adsorption of atrazine onto a luvisol soil amended with sewage sludge. Concentration measurements by ELISA and MEKC, are shown.

	K_F (mg kg^{-1} (mg L^{-1})$^{-N}$)	N	R^2
ELISA	1.6 ± 0.2[b]	0.70 ± 0.09	0.977
MEKC	1.6 ± 0.1	0.72 ± 0.04	0.993
t Student[a]	0.30	0.32	

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

3.3. Adsorption experiment

The Freundlich parameters (K_F and N) were calculated from the fitting of a non-linear regression according to the equation $Q_e = K_F \times C_e^N$ to the experimental data, where Q_e is the total sorbed mass 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. Adsorption isotherms were obtained by plotting the amount of atrazine sorbed per unit weight of soil at equilibrium (Q_e) versus the amount of chemical per volume of solution at equilibrium (C_e).

The Freundlich equation is able to reasonably describe the adsorption of atrazine on the soil studied ($R^2 > 0.97$). The Freundlich adsorption coefficient is an empirical constant of the Freundlich model expressing soil sorbent capacity (sorption isotherm slope) for the studied range of sorbate concentration and the higher the value, the stronger is adsorption. The low K_F value obtained (Table 2), using both methods, reflects low adsorption capacity and is commonly associated with greater permeability of the soil leading to a higher leaching potential [7]. The sorption isotherm is non-linear, showing N values smaller than 1, indicating that the percentage of atrazine adsorbed to the soil decreased as the initial concentration increased.

A Student *t*-test was applied to compare the results obtained by both methods. Since the *t* calculated presented lower values for both K_F and N than the critical *t* value for 4 degrees of freedom at 95% confidence level, it can be stated that there are no significant differences between the results obtained by ELISA and MEKC.

4. Conclusions

It has been shown that ELISA can be a valid alternative in assaying concentrations from sorption experiments of organic pollutants onto soils. Atrazine was determined by immunoassay using a polyclonal antibody after selection of an appropriate structure for synthesizing a tracer that produces the signal of the assay. Since selectivity against other structurally similar compounds is not an issue with laboratory model experiments and sensitivity is not crucial in sorption experiments – starting concentrations can be widely adapted to the measurement range of the analytical method – we focused on matrix effects of co-extracted soil compounds of a high organic carbon soil and found that dilution by a factor of 1000 usually will overcome matrix interferences in ELISA. For other instances or if the adsorption isotherm should be assessed in low concentration ranges, a solid-phase extraction procedure was conceived to successfully clean up samples before ELISA analysis.

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References

[1] S. Reichenberger, M. Bach, A. Skitschak, H.G. Frede, Sci. Total Environ. 384 (2007) 1–35.
[2] I. Baranowska, H. Barchanska, R.A. Abuknesha, R.G. Price, A. Stalmach, Ecotoxicol. Environ. Saf. 70 (2008) 341–348.
[3] W. Mersie, C. Seybold, J. Agric. Food Chem. 44 (1996) 1925–1929.
[4] M.C. Smith, D.R. Shaw, J.H. Massey, M. Boyette, W. Kingery, J. Environ. Qual. 32 (2003) 1393–1404.
[5] G.G. Ying, R.S. Kookana, M. Mallavarpu, Environ. Pollut. 134 (2005) 71–77.
[6] I.D. Kovaios, C.A. Parakeva, P.G. Koutsoukos, A.C. Payatakes, J. Colloid Interface Sci. 299 (2006) 88–94.
[7] F.V. Correia, A. Macrae, L.R.G. Guilherme, T. Langenbach, Chemosphere 67 (2007) 847–854.
[8] G.-C. Chen, X.-Q. Shan, Y.-Q. Zhou, X.-e. Shen, H.-L. Huang, S.U. Khan, J. Hazard. Mater. 169 (2009) 912–918.
[9] J.B. Sass, A. Colangelo, Int. J. Occup. Environ. Health 12 (2006) 260–267.
[10] J. Lu, Y. Li, X. Yan, B. Shi, D. Wang, H. Tang, Colloids Surf. A: Physicochem. Eng. Aspects 347 (2009) 90–96.
[11] M. Kah, C.D. Brown, Chemosphere 68 (2007) 1335–1343.
[12] H.M. Lesan, A. Bhandari, Water Res. 37 (2006) 1644–1654.
[13] L. Nemeth-Konda, G. Füleky, G. Morovjan, P. Csokan, Chemosphere 48 (2002) 545–552.
[14] J.P. Gao, J. Maguhn, P. Spitzauer, A. Kettrup, Water Res. 32 (1998) 1662–1672.
[15] A. Boivin, R. Cherrier, M. Schiavon, Chemosphere 61 (2005) 668–676.
[16] R.D. Wauchope, S. Yeh, J.B.H.J. Linders, R. Kloskowski, K. Tanaka, B. Rubin, A. Katayama, W. Kördel, Z. Gerstl, M. Lane, J.B. Unsworth, Pest Manage. Sci. 58 (2002) 419–445.
[17] L.B.O. dos Santos, G. Abate, J.C. Masini, Talanta 68 (2005) 165–170.
[18] OECD, Guideline TG 106, OECD Guideline for the Testing of Chemicals, Adsorption–Desorption using a Batch Equilibrium Method, OECD (Organization for Economic Co-operation and Development), Paris, 2000.
[19] M.K. Amistadi, J.K. Hall, E.R. Bogus, R.O. Mumma, J. Environ. Sci. Health B 32 (1997) 845–860.
[20] B. Chefetz, Y.I. Bilkis, T. Polubesova, Water Res. 38 (2004) 4383–4394.
[21] Y. Drori, Z. Aizenshtat, B. Chefetz, Soil Sci. Soc. Am. J. 69 (2005) 1703–1710.
[22] J.H. Park, Y. Feng, S.Y. Cho, T.C. Voice, S.A. Boyd, Water Res. 38 (2004) 3881–3892.
[23] M.M. Socías-Viciana, M. Fernández-Pérez, M. Villafranca-Sánchez, E. González-Pradas, F. Flores-Céspedes, J. Agric. Food Chem. 47 (1999) 1236–1241.
[24] M. Ben-Hur, J. Letey, W.J. Farmer, C.F. Williams, S.D. Nelson, Soil Sci. Soc. Am. J. 67 (2003) 1140–1146.
[25] O.S. Mbuya, P. Nkedi-Kizza, K.J. Boote, J. Environ. Qual. 30 (2001) 71–77.
[26] F. Prata, A. Lavorenti, J. Vanderborght, P. Burauel, H. Vereecken, Vadose Zone J. 2 (2003) 728–738.
[27] D.L.D. Lima, G.L. Erny, V.I. Esteves, J. Sep. Sci. 32 (2009) 4241–4246.
[28] G.K. Stearman, M.J.M. Wells, Bull. Environ. Contain. Toxicol. 51 (1993) 588–595.
[29] R.J. Bushway, B. Perkins, S.A. Savage, S.J. Lekousi, B.S. Ferguson, Bull. Environ. Contam. Toxicol. 40 (1988) 647–654.
[30] E.M. Thurman, M. Meyer, M. Pomes, C.A. Perry, A.P. Schwab, Anal. Chem. 62 (1990) 2043–2048.
[31] B. Gruessner, N.C. Shambaugh, M.C. Watzin, Environ. Sci. Technol. 29 (1995) 251–254.
[32] J. Gascón, G. Durand, D. Barceló, Environ. Sci. Technol. 29 (1995) 1551–1556.
[33] T.S. Lawruk, C.E. Lachman, S.W. Jourdan, J.R. Fleeker, D.P. Herzog, F.M. Rubio, J. Agric. Food Chem. 41 (1999) 747–752.
[34] H.W. Scherer, H.E. Goldbach, J. Clemens, Plant Soil Environ. 49 (2003) 531–535.
[35] A. Frey, B. Meckelein, D. Externest, M.A. Schmidt, J. Immunol. Methods 233 (2000) 47–56.
[36] C. Wittmann, B. Hock, Food Agric. Immunol. 1 (1989) 211–224.
[37] L. Weil, R.J. Schneider, O. Schäfer, P. Ulrich, M. Weller, T. Ruppert, R. Niessner, Fresenius J. Anal. Chem. 339 (1991) 468–469.
[38] R.J. Schneider, L. Weil, R. Niessner, Int. J. Environ. Anal. Chem. 46 (1992) 129–140.
[39] R.J. Schneider, L. Weil, R. Niessner, Fresenius J. Anal. Chem. 343 (1992) 145–146.
[40] M.G. Weller, R. Niessner, SPIE Proc. Ser. 3105 (1997) 341–352.
[41] M.G. Weller, L. Weil, R. Niessner, Mikrochim. Acta 108 (1992) 29–40.
[42] P. Ulrich, M. Weller, L. Weil, R. Niessner, Vom Wasser 76 (1991) 251–266.
[43] P. Schneider, B.D. Hammock, J. Agric. Food Chem. 40 (1992) 525–530.
[44] R.A. Dudley, P. Edwards, R.P. Ekins, D.J. Finney, I.G. McKenzie, G.M. Raab, D. Rodbard, R.P. Rodgers, Clin. Chem. 31 (1985) 1264–1271.
[45] J. Kaur, R.C. Boro, N. Wangoo, K.R. Singh, C.R. Suri, Anal. Chim. Acta 607 (2008) 92–99.
[46] P. Fruhstorfer, R.J. Schneider, L. Weil, R. Niessner, Sci. Total Environ. 138 (1993) 317–328.
[47] R.P. Ekins, Ligand Quart. 4 (1981) 33–44.
[48] D.L.D. Lima, S.M. Santos, H.W. Scherer, R.J. Schneider, A.C. Duarte, E.B.H. Santos, V.I. Esteves, Geoderma 150 (2009) 38–45.
[49] A. Deng, M. Himmelsbach, Q.-Z. Zhu, M. Sengl, W. Buchberger, R. Niessner, D. Knopp, Environ. Sci. Technol. 37 (2003) 3422–3429.