Design of experiments in environmental chemistry studies: Example of the extraction of triazines from soil after olive cake amendment

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ABSTRACT

The disposal of natural, composted and vermicomposted olive cake for modifying the fate of triazine herbicides with different physicochemical properties (terbuthylazine, cyanazine, simazine and prometryn) has been tested. Experimental design (surface response methodology plus desirability function) to multicriteria optimization was carried out to evaluate both dose and type of amendment to retain the cited analytes and to develop two simple and low cost analytical methods for extracting triazines from soil. From a methodological point of view, classical and D-optimal designs were employed depending on the problem. Thus, the best combination of soil amount and solvent ratio, the most important parameters affecting triazine extraction from soil, was looked for by means of Central Composite Designs. Under the optimized conditions, the range of triazines recovery was 75–85% for shaking extraction and 87–107% for ultrasonic extraction. Regarding the amendment assay, D-optimal design was selected to keep the reliability of the estimations. Natural olive cake added to the soil at a high dose (8%) reduced herbicide recovery to ca. 50–60% for terbuthylazine, prometryn and simazine, while cyanazine recovery was negligible. Design of experiment provides an efficient working strategy to explore those conditions which ensure the optimum or target value of several responses evaluated simultaneously.

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1. Introduction

Today, two strongly promoted environment management strategies for the developing countries are urban agriculture and reduction or reuse of solid waste. So by-products either from industrial, urban or agricultural activities have been applied to enhance the fertility of soils poor in organic matter, such as those currently found in the Mediterranean region, and to modify the fate of potential pollutants, such as triazine herbicides [1–3]. In Andalusia the olive culture is of high socioeconomic importance constituting between 60 and 80% of the total national production, which represents about 2.44×10^3 ha. Therefore, olive cake or alperujo from the continuous centrifugation two-phase process is generated in huge amounts, ca. $3.5\,\mathrm{Mg\,y^{-1}}$.

The disposal of olive cake has been regarded as a serious environmental problem, different approaches for its exploitation being envisaged, such as the use of biomass to produce electricity [4] or ethanol [5]. Olive cake, natural, composted or vermicomposted could be also applied for bioremediating purposes in soils contaminated with pesticides [6].

Environmental studies generate data to assess pollutant hazards and risks to users and consumers. At the heart of these studies lie analytical data from soil samples, whose accuracy and reliability are of increasingly importance to make technically and administratively correct decisions for a stated purpose. A new frame of integration between analytical procedures and chemometric methods has made the extraction of relevant underlying analytical information possible. Design of experiment (DOE) is a tool within Chemometrics, which allows extracting chemically relevant information via experiment optimization, data processing, calibration, quality control and organization of the analytical process, largely applied in the environmental science [7–9] and other life sciences [10] where data interpretation is of great interest.

Both qualitative and quantitative analyses conclude a procedure of sample preparation. The extraction step is the least evolved part of most analytical procedures. The collection of experimental factors with an influence on it, known as the "analytical system", is therefore, responsible for the accuracy of analytical data. This is the aim of the application of DOE undertaken in analytical chemistry.

In the last decade there has been an increasing demand for new extraction techniques, amenable to automation with shortened extraction times and reduced organic solvent consumption preventing pollution in analytical laboratories and reducing sample preparation cost. Driven by these purposes advances in sample

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preparation have resulted in a number of techniques such as MAE, SFE or PLE [9,11–13] that provide the possibility of working at elevated temperatures and pressures, which drastically improve the speed of the extraction process. The latter two techniques, which use supercritical fluids or solvents at a subcritical state, shift the equilibrium constant when compared to conventional solvent extraction methods [12]. The similarity between these techniques is the moderate high investment required [14].

On the contrary, traditional techniques for solid matrices, such as the well-known Soxhlet extraction, together with shaking and sonication [15,8], which can also provide efficient extractions with low investment, seem to demand decreasing attention due to their drawbacks: long extraction times (especially for Soxhlet), relative high solvent consumption, occasional need of a clean-up step and possible repeated extractions in the case of sonication. Two of these methods, less in fashion in recent periods but of low cost, are the object of the proposed examples.

In summary, this paper presents an application of experimental designs as an adequate tool to make the environmentalist and analyst task easier and quicker and provide more reliable results to the environmental questions posed. First, simple and low cost extraction methods, shaking and ultrasonic, were optimized for some triazine herbicides in soil samples; second, an environmental application focused on the olive agricultural by-products as pesticide soil bioremediation measures was qualitative and quantitatively assessed. Examples were selected to represent a classical design and a D-optimal design.

2. Experimental

2.1. Herbicides and reagents

Terbuthylazine (Tb), cyanazine (Cz), simazine (Sm) and prometryn (Pm), all with purity >99% (Dr. Ehrenstorfer, Germany) were used. Milli Q water (Millipore, MA, USA) and residue analysis quality solvents (methanol, hexane, toluene) (Merck, Darmstadt, Germany) were employed.

2.2. Soil and amendments

The upper layer (0–25 cm) of a soil from an olive orchard located near Iznalloz (Granada province, Southern Spain) was sampled, air-dried and sieved through 2 mm. The soil is a Calcaric Regosol, with silty clay loam texture, 34% clay, 56% silt and 10% sand; containing 44.1% $CaCO_3$, 2.2% organic carbon and pH (5/20 soil/water ratio) 7.9. Olive cake (A), compost (C) and vermicompost (V) from olive cake, previously sieved through 2 mm sieve, were added to the soil at 2, 4 and 8% (w/w). The olive cake was provided by an olive oil industry (Romeroliva, Granada, Spain). The mature compost and vermicompost were prepared as described in Delgado-Moreno et al. [16].

2.3. Extraction procedure and analysis

In glass vials with screw caps fitted with PTFE seals, soil samples with 20% moisture content were spiked with an aqueous herbi-cide mixture to provide a final herbicide concentration of 1 $\mu g\,g^{-1}$, which corresponds to the usual land application rate. The soil samples were left to stand overnight at $15 \pm 0.1\,^\circ C$ in an incubating chamber (Hotcold M, Selecta). Then the extraction solution (methanol) was added. The samples were homogenized in a vortex mixer and extracted in a bath by ultrasound-assisted extraction (UAE) (200w, Ultrasons, Selecta 1) or by shaking in an end-over-end mixer (Reax2, Heidolph). The extraction conditions, including soil weight, extraction solvent volume and extraction time are summarized in Table 1. Then the samples were centrifuged at 3000 rpm and 15 $^\circ C$ for 15 min (5810 R, Eppendorf) and the supernatant was filtered through a filter paper. The vial and the filter paper were additionally washed with 2 × 2 mL methanol. Finally the extract was concentrated to dryness in a rotary evaporator (Laborota 4000, Heidolph) at 60 $^\circ C$. The dry residue was dissolved with 2 mL of hexane:toluene (1:1).

Samples were analyzed by gas chromatography in a Varian Star 3400 CX, equipped with a thermionic specific detector and an 8200 automatic injector (all from Varian, Madrid, Spain) [16]. The analytes were quantified by linear calibration at five concentrations levels in the range 2–20 mg L^{-1}.

3. Experimental designs

3.1. Extraction method

A full factorial 2^2 design was developed to optimize the factors related to the extraction procedure to assure quantitative extraction for each herbicide: methanol volume and soil amount, whereas the extraction time was set at fixed values (Table 1). All experiments were performed in randomized order to minimize the effects of uncontrolled factors that may introduce a bias on the measurements. The data correspond to the average of two independent injections of each solution. The replicates at the centre point are carried out in order to achieve a near orthogonal design and to estimate the pure error variance. This ensures independent estimates of the model parameters.

This design allowed us to obtain the surface response, fitting the data to the following first-order polynomial model (Eq. (1)):

$$Y = \beta_0 + \beta_1 s + \beta_2 \mathrm{me} + \beta_{12} s * \mathrm{me} + \varepsilon$$

where Y is the herbicide recovery, s the soil amount, me the methanol volume, β_i the estimated model coefficients and ε the experimental error.

When the linear model shows a significant lack-of-Fit (LoF) together with a strong curvature, a second-order polynomial model is necessary for estimating the experimental response and finding the optimal point (Eq. (2)):

$$Y = \beta_0 + \beta_1 s + \beta_2 \mathrm{me} + \beta_{11} s^2 + \beta_{22} \mathrm{me}^2 + \beta_1 s * \mathrm{me} + \varepsilon$$

To fit the second-order model, the factorial design was complemented with a central composite design that includes the full factorial 2^2 and points centred on the faces (CCF). The experimental domain is shown in Table 1 and the design matrices in Table 2.

Table 1
Experimental domain for CCF designs

	Agitation extraction			Ultrasonic extraction		
	Level (−)	Level (+)	Optimum	Level (−)	Level (+)	Optimum
Soil (s) (g)	10	20	15	5	15	10
Extractant (me) (mL)	15	40	36	30	60	50
Time fixed (h)			24			1/4

Table 2
Design matrices for the three experimental sets

Run	Agitation extraction		Ultrasonic extraction		Soil remediation	
	s (g)	me (mL)	*s* (g)	me (mL)	Material	Dose (%)
1	10	15	5	30	A	2
2	20	15	15	30	A	2
3	10	40	5	60	V	2
4	20	40	15	60	V	2
5	15	27.5	10	45	C	2
6	15	27.5	10	45	C	2
7	15	27.5	10	45	A	4
8	10	27.5	5	45	V	4
9	20	27.5	15	45	C	4
10	15	15	10	30	A	8
11	15	40	10	60	A	8
12	15	27.5	10	45	V	8
13			**8**	**30**	V	8
14			**8**	**40**	C	8
15			**15**	**50**	C	8
16			**10**	**50**	C	8
17			**8**	**60**	C	8
18					C	8

s: soil amount. me: methanol volume. A, V, C: olive cake, vermicompost and compost, respectively. In bold, test points.

3.2. Soil amendment experiment

The design proposed is a D-optimal design, more flexible than the classical symmetric ones (factorial, central composite, etc.), and may, therefore, better adapt to those cases in which either any combination of values in the experimental variables is not possible [17], the number of experiments is limited [18] or, as in our case, the experimental region is not regular in shape [19]. The D-optimal design procedures provide a set of experimental conditions where the factor effects are maximally independent from each other (will give the maximum amount of information from the experimental region) given a specific expected type of model will fit the data.

Three forms of olive cake (natural, composted and vermicomposted) were applied at 3 doses (2, 4 and 8%) to soil in order to improve retention of the four herbicides cited above. The UAE proposed method was used to extract the herbicides from soil.

With these two factors and assuming a second-order model we generated a D-optimal design through the exchange algorithm, so that matrix determinant of the associated dispersion matrix $(X'X)^{-1}$ is minimized, where X is the model matrix with dimensions Nxp (*N* the number of experiments and *p* the number of model coefficients). The design consists of 18 experiments (Table 2) showing a *G*-efficiency of 96% and a condition number of 4.9. The latter, calculated as the ratio of the largest and the smallest eigenvalues of the X'X matrix, indicates the degree of multicollinearity present in the design matrix. The *G*-efficiency value is a measure of the statistical performance of the design in relation to that of a fractional factorial design (for which *G*-efficiency = 100%), and a value above 65–70% has been recommended [20]. A condition number of 1 indicates a perfect design, but most designs are found in the range 1–5. Thus the design meets the requirements.

3.3. Data evaluation

We will here use three evaluation tools for giving guidance of how to formulate the most valid model. First, the two statistics, goodness of fit, R^2 and goodness of prediction Q^2 are used to test the right model. These should be as close to unity as possible, and preferably not separated by more than 0.2–0.3 [20]. As a guide to data of chemical nature [21], values of $R^2 > 0.8$ and $Q^2 \geq 0.5$ are acceptable and of $Q^2 > 0.8$ is considered excellent.

The second tool is the evaluation of the model residuals using a normal probability plot (*N*-plot) for detecting outlier experiments and for supporting the adequacy of the model if the plot is a straight line. If there are important deviations, this may indicate an inappropriate model, the need for a transformation or errors in the data.

If none of the model assumptions were violated, the analysis of variance, ANOVA, is examined. It checks the adequacy of the regression model in terms of LoF test and whether the coefficients estimated are actually significant. The LoF test implies the separation of the residual response sum of squares into the components model error and the pure error, and their sizes compared by an *F*-test. The fitted model is considered adequate if the *F*-test is significant ($P < 0.05$). Next, the significance of regression coefficients estimated was considered, deleting those with a negligible effect on the response at a significance level of 95%.

Additionally, the reproducibility of the experiments made under the same conditions is computed. If it is referred to the centre points, the mean value of these responses is another estimate of the constant term in the model. Significant differences when compared with the estimated (β_0) by the model indicate a curvature, i.e., the linear model is unable to fit the data. Provided that the underlying regression model has been found valid, the visualization of the modelling results in terms of coefficients, contour or response surface plots, is recommended. This facilitates the understanding of the model and also helps in decision-making.

The validity of the model in the case of development of UAE, i.e., how well the model is going to perform on future as-yet-unseen data, is tested by four test points (Table 2).

The identification of the optimal extraction factor settings which optimize the four responses simultaneously is found with an optimization routine based on the desirability function approach, such as the Derringer function [11]. The procedure involves creating a desirability function for each individual response (d_i), a partial desirability function, whose values range from 0 (undesirable recovery) to 1 (completely acceptable recovery). Then an overall desirability function (*D*), maximized choosing the best conditions of the extraction variables, is defined as the weighed geometric mean of the partial desirability functions. A high value of *D* is obtained only if all individual desirabilities (d_i) are high. The values of *D* computed from the observed responses allow us to locate an optimal region.

The software package Modde 7.0 (Umetrics, Sweden) was used to create experimental designs and to analyze the experimental data and NEMROD-W (Marseille, France) was used to compute the desirability function.

4. Results and discussion

4.1. Optimization of the soil/solvent ratio in the shaking method

Seven experiments were carried out according to the conditions fixed by the experimental design shown in Table 2 (runs 1–7). Shaking time was fixed to 24 h since this is a commonly reported extraction period [22,23]. ANOVA of the results indicated that the data did not fit to a linear model, since it presented LoF (*P* values are 0.041 (Sm), 0.034 (Tb), 0.023 (Pm) and 0.029 (Cz)) and curvature. Thus this design was not appropriate and was complemented (Table 2, runs 8–12) to a second-order model, that is, a CCF. Experimental data were fitted to the second-order model by least squares and the significance of the model and estimated regression coefficients are reported in Table 3 for all the responses. The values of the parameters for testing the adequacy of the model together with the residual plots confirmed the assumption that the quadratic model was correct. There was no LoF ($P > 0.05$), i.e., the data described well

Table 3
Significant regression coefficients (scaled and centered) and fitting parameters for the surface response obtained with $Y = \beta_0 + \beta_1 s + \beta_2 me + \beta_{11} s^2 + \beta_{22} me^2 + \beta_{12} s*me + \varepsilon$

Method	Herbicide	β_0	β_1	β_2	β_{11}	β_{22}	β_{12}	R^2	Q^2	Reproducibility
Shaking	Simazine	64.94	−4.58	12.32	−4.66	ns	ns	0.953	0.741	0.906
	Terbuthylazine	65.29	−5.47	11.31	−4.07	ns	ns	0.973	0.867	0.929
	Prometryn	69.67	−3.66	16.37	−2.38	ns	ns	0.962	0.834	0.981
	Cyanazine	75.40	−7.96	6.99	−7.38	ns	ns	0.919	0.665	0.862
UAE	Simazine	96.11	−6.82	5.90	−4.20	n.s.	n.s.	0.919	0.903	0.879
	Terbuthylazine	93.97	−10.83	10.73	n.s.	−8.45	3.80	0.952	0.939	0.943
	Prometryn	100.46	−9.61	10.18	ns	−4.92	5.24	0.856	0.783	0.821
	Cyanazine	108.37	−6.79	6.82	−7.15	n.s.	7.00	0.868	0.826	0.857

ns: not significant at 0.05 level.

the model chosen and the predictive knowledge of the model was statistically correct; $R^2 > 0.91$ indicated that the model explained the experimental variability found in the recoveries; $Q^2 > 0.66$ indicated the excellent predictive ability of the model; besides the difference between R^2 and Q^2 is less than 0.3 as reported [20]; the reproducibility showed that the error of the model was in the same range as the pure error. As can be seen (Table 3) both main factors were found to be significant for all compounds but interaction $s*me$ were not significant (β_{12}, $P > 0.05$).

It is evident that solvent volume is the most significant factor in the extraction process, an increase of methanol volume increases linearly (β_2, $P < 0.05$; β_{22}, $P > 0.05$) herbicide recovery. On the other hand, soil weight has a quadratic and negative effect on the recovery of all herbicides (β_{11}, $P < 0.05$), which behaved similarly to changes in extraction parameters.

The contour plot (Fig. 1) shows that the most efficient extraction conditions can be reached by using 15 g of soil and methanol volumes greater than 30 mL.

Nevertheless, the most favorable extraction parameters were reached by simultaneously maximizing the response surface for the four herbicides recoveries, with an overall desirability (i.e., D) function, calculated considering 70% as the threshold below which

the analytes recoveries were not considered acceptable. Our target value was a recovery rate close to 85% for all the compounds. All partial desirability functions were similarly weighed to 1. Thus, the conditions providing the maximum desirability value ($D = 0.75$) are a methanol volume of 36 mL and a soil amount of 15 g, as can be seen in Fig. 2, where the regions in grey correspond to $D = 0$ and the contour lines represent D values close to 1. The individual recoveries corresponding to the latter value of D are: Sm 76% ($d_1 = 0.78$); Tb 75% ($d_2 = 0.46$); Pm, 85% ($d_3 = 0.99$); and Cz 79% ($d_4 = 0.87$).

The recoveries encountered for the four triazines can be explained by the different solubility in aliphatic alcohols, in particular in ethanol [24]. Tb, whose recovery was the lowest followed by Sm, has a solubility in ethanol of $14\,g\,L^{-1}$, whilst that of Cz and Pm was 45 and $140\,g\,L^{-1}$, respectively. Sm and Tb are also less soluble in other polar solvents, like acetone, than the other two herbicides.

4.2. Development of the ultrasound extraction method

A duplicate two-level full factorial design, 2^2, involving 8 runs, was used as a first approach to generate the response surface of the UAE process (Table 2, runs 1–8). The maximum and minimum levels
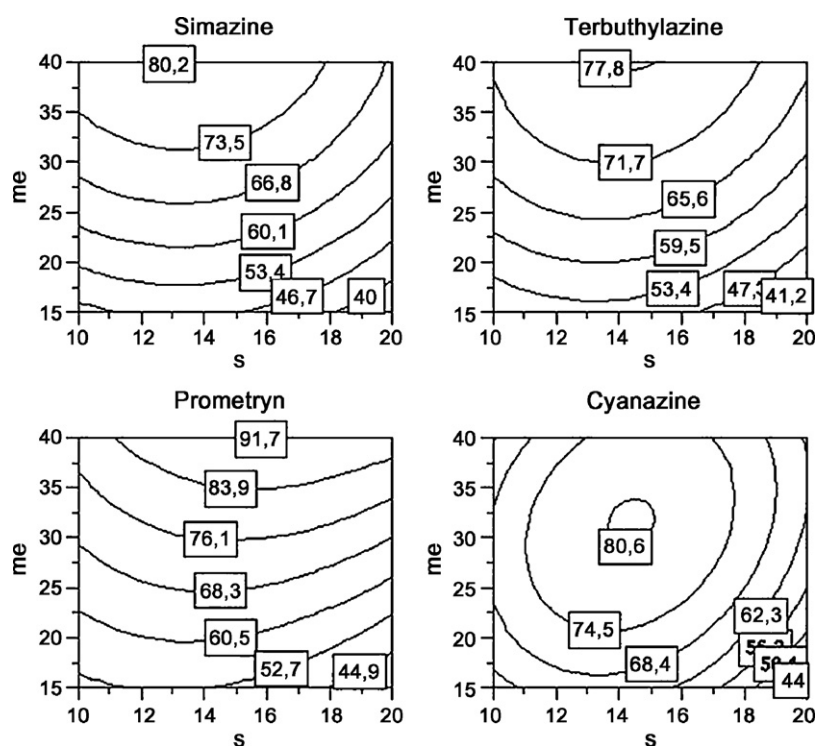

Fig. 1. Contour plots from the central composite design for the recovery of individual herbicides extracted by the shaking method (*s*: weight of soil; *me*: methanol volume).
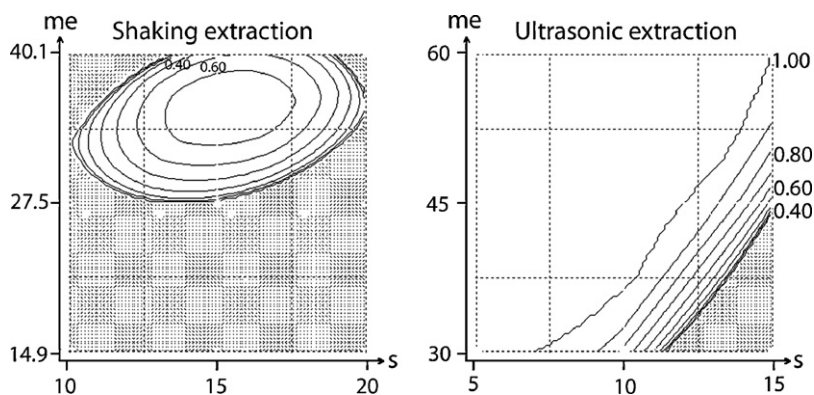
Fig. 2. Contour plot of the overall desirability functions in the space of the factors: soil amount (*s*) and methanol volume (me). The grey zone corresponds to zero desirability, and contour lines to several % of the overall desirability function.

of extraction variables that defined the experimental domain were chosen according to previous experience or limited by the material employed, i.e., maximum volume of methanol. One precaution was the temperature reached in the solution during the extraction process, although these compounds have been reported to be quite stable to heating [24]. The highest temperature measured was 50 °C, a safe value to avoid decomposition. Therefore, an extraction time of 15 min was selected. Duplication of the design increases its power to detect effects and improves the precision of the esti-

mated effects by a factor of $\sqrt{2}$. This alternative is often taken in the presence of response measurement variability, as occurs with herbicides recoveries by UAE. Experimental data were fitted to Eq. (1) by least squares. The ANOVA shows a significant LoF ($P = 0.013$ for Tb and 0.027 for Cz) which suggests that data did not fit to the linear model. Therefore, the design was completed to fit a central composite design (Table 2, runs 9–12) and data were fitted to the second-order model (Eq. (2)), whose significance and regression coefficients are reported (Table 3). Because of the high R^2 and Q^2
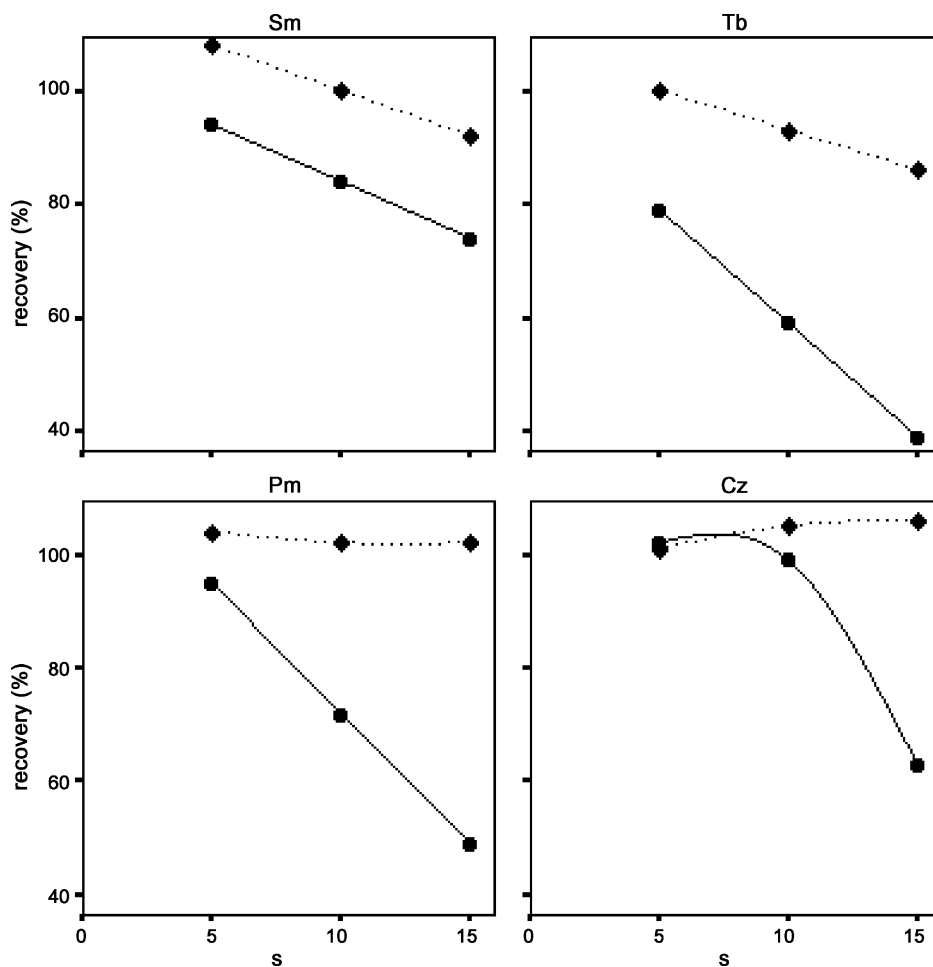
Fig. 3. Extraction volume*soil weight interaction plots for all compounds using UAE method: (●) 10 mL and (♦) 60 mL of methanol (Sm: simazine; Tb: terbuthylazine; Pm: prometryn; Cz: cyanazine).

values, the conclusion is that the quadratic model is well founded and valid. Also the LoF test and the *N*-plot of the model residuals (figure not shown) strongly point in the direction of a valid model. Furthermore, interaction between extractant volume and soil amount (s*me) was significant (β_{12}, $P < 0.05$) for Tb, Pm and Cz. Thus, a higher soil weight will induce a greater decrease of herbicide recovery, i.e., up to 40% for Tb, if extraction is made with 10 mL (Fig. 3). On the contrary, if 60 mL of methanol is used for extraction, soil amount slightly affects the recoveries of Tb and Sm, but does not remarkably alter Pm and Cz recoveries.

As it happened with the shaking extraction procedure, this behavior can be attributed to the lower solubility of the former herbicides in the extraction solvent. However, higher recoveries are obtained with the ultrasonic extraction with regard to the shaking process, because it is a more vigorous extraction system than simple contact between solid and liquid phases.

The UAE method was optimized with the same desirability procedure as in Section 4.1. Thus, the conditions producing the maximum desirability value ($D = 1$) are a methanol volume of 50 mL and a soil amount of 15 g. The two-dimensional plot of the global desirability obtained is shown in Fig. 2. The individual recoveries, all with $d_i = 1$ are: Sm, 91%, Tb 87%, Pm, 101% and Cz 107%. Under other circumstances, i.e., soil samples with higher herbicide content, less soil could be extracted to adjust to chromatographic sensitivity, for instance, 5/10 g soil. The model predicts a *D* value of 0.963 ranging the herbicide recoveries from 80 to 100%.

To assure the validity of the experimental results four additional test points were compared with the values predicted by the model. A significant statistically linear regression $Y_{\text{experimental}} = 18.8 + 0.840 \times Y_{\text{predicted}}$ ($R^2 = 0.815$) was obtained, with the intercept not significantly different from zero ($\sigma_{\text{intercept}} = 9.70$; $P = 0.090$) and the slope statistically equal to 1 ($\sigma_{\text{slope}} = 0.10$; $P = 0.001$), which indicated that the predicted and experimental values were not statistically different. UAE was then selected due to higher recoveries (87–107% instead of 75–85%)

Table 4
Recoveries of pesticides from soil samples spiked at three levels and extracted with the UAE method, $n = 6$ (R.S.D.)

	0.75 µg g^{-1}	1.5 µg g^{-1}	3 µg g^{-1}
Simazine	97.2 (7)	84.2 (8)	95.6 (7)
Terbuthylazine	90.8 (4)	86.5 (7)	88.7 (9)
Prometryn	91.9 (4)	93.2 (7)	90.9 (9)
Cyanazine	96.0 (5)	95.3 (8)	92.4 (10)

and because it was more rapid, allowing the extraction of the four herbicides in a single stage.

4.3. Performance evaluation of the UAE method

Within-run precision ($n = 6$) oscillated between 2 and 3% and between 3 and 10% for standard and soil samples, respectively. Among run precision ($n = 4$) ranged, for standard and extracted soil samples, from 3 to 8% and from 7 to 12%, respectively.

Difficulties were found to validate the method using Certified Reference Material due to the lack of natural matrix certified materials containing relevant pesticides in soil, because these materials are difficult and expensive to characterize, store and maintain [25]. Therefore, the method was validated with fortified soil samples at two additional spiked analyte concentrations. The results (Table 4) show the successful application of this UAE extraction method for triazine herbicides in soil samples over the range 0.75–3 µg g^{-1}.

4.4. Evaluation of the reuse of olive agricultural by-products

The present study was evaluated by a duplicate D-optimal design (Table 2). The partial least square (PLS) regression of experimental data produced a quadratic model with $R^2 = 0.915$ (Sm), 0.881 (Tb), 0.852 (Pm), 0.946 (Cz) and $Q^2 = 0.854$ (Sm), 0.803 (Tb), 0.753 (Pm), 0.913 (Cz), upon removal of non-significant terms ($P > 0.05$),
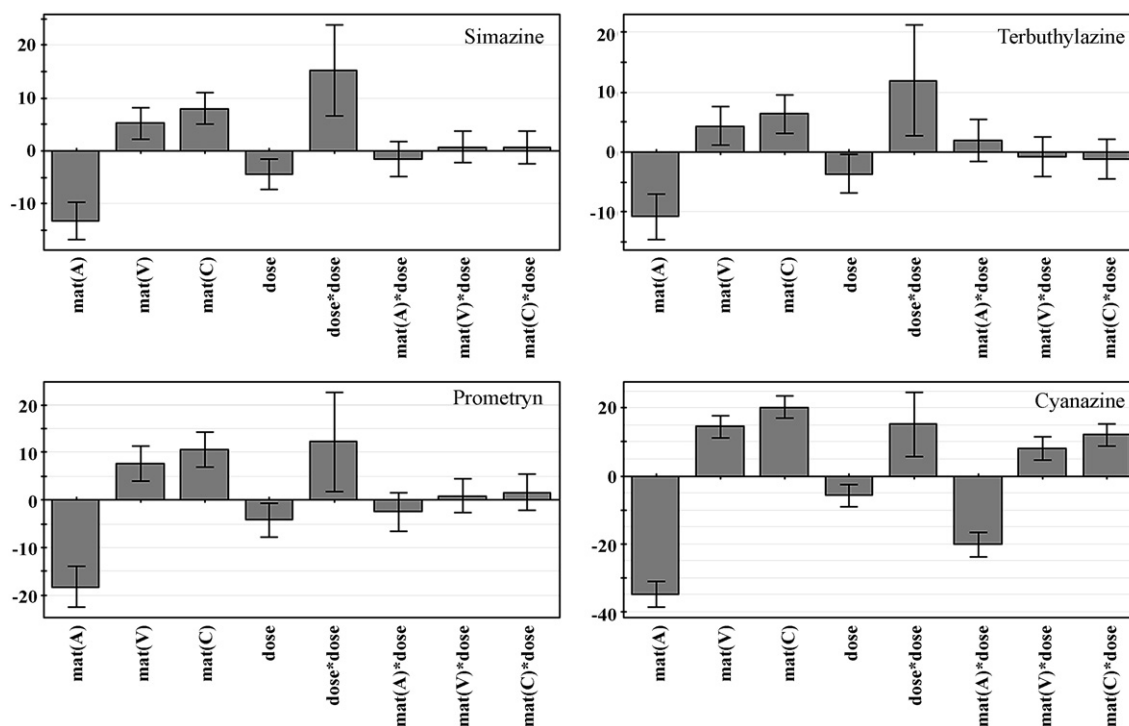

Fig. 4. Bar chart with scaled and centred regression coefficients with 95% confidence intervals of D-optimal design for the bioremediation experiment (A: olive cake; C: compost; V: vermicompost).
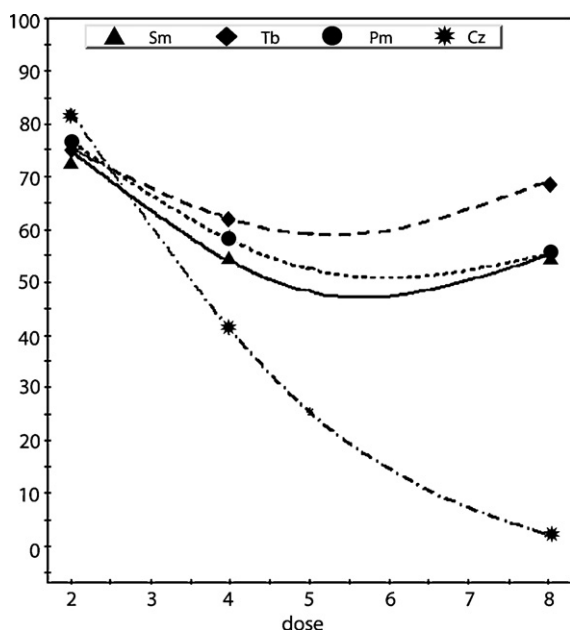
Fig. 5. Effect of the addition of olive cake on the retention of all herbicides onto the soil (Sm: simazine; Tb: terbuthylazine; Pm: prometryn; Cz: cyanazine).

suggesting a sound quadratic model. This model did not show a LoF ($P < 0.05$) and presented normally distributed residuals.

The regression coefficients of the model, displayed in Fig. 4, show that the effect of the addition of natural olive cake and the quadratic term of the added dose are the most meaningful factors. In the case of Cz, all effects are significant ($P < 0.05$) making this herbicide more vulnerable to the extraction systems in the presence of the soil amendments. The sorption–desorption behavior of triazine herbicides in amended soil showed that natural olive cake induced a considerable enhancement of herbicide sorption [16]. Natural olive cake should be added to the soil at a high dose (8%), in order to increase the herbicides retention onto the soil. For this amendment herbicide recovery was reduced to ca. 50–60% for Tb, Pm and Sm, while Cz recovery was negligible which is an indication of the bioremediation efficacy of this agricultural by-product (Fig. 5). However, the use of natural olive cake has to be considered with care because of its relatively high content in polyphenols and organic acids [26], related to phytotoxic and antimicrobial effects [27]. To solve this problem, the addition of composted or vermicomposted alperujo would retain the herbicides to a lesser extent (Fig. 5), but with a decreased toxicity because of a reduction in the polyphenols concentration [28]. Compost and vermicompost of different organic wastes have been presented as an alternative for the bioremediation of pesticide-contaminated soil [29,6].

5. Conclusions

The current study certainly presents examples of experimental design using response surface methodology (RSM) in environmental systems, encouraging the elaboration of future studies conducted on the same basis.

The importance of DOE is obvious because much of what is learnt about the environment is based on chemical data. The application of experimental design approach is an efficient means of optimization of sample treatments, such as those presented here: ultrasound assisted and shaking procedures. The CCF designs are adequate to evaluate how soil and extractant amounts influence the extraction of four herbicides from a fortified soil with only 12 experimental runs, finding those extractions conditions which jointly assure

good accuracy. Besides it provides additional information which cannot be obtained with other experimental setups: it identifies the meaningful parameters, the existence of interactions, it makes predictions and gives results together with their quality parameters.

Additionally, the optimal UAE extraction parameters prove that the extraction of the herbicides can be done in a single stage which allows reducing not only the overall analysis time and consequently the cost, but also the use of organic solvents.

The D-optimal design in the bioremediation example provides an experimental design using RSM in environmental systems, encouraging the elaboration of future studies conducted to decrease variability and quality improvement of the conclusions.

In this paper, we have focused on practical aspects of design applications, and in doing so proposed an efficient working strategy, which benefits from chemometric analysis tools. As illustrated by the examples, the analysis of data with DOE is simple, straightforward, and works well in reality.

Acknowledgements

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