

Research article

Optimization of a microwave-assisted extraction method for the analysis of the persistent organic pollutant *p,p'*-DDT in domestic sewage sludge

Darlan Ferreira Silva, Maria Diva Landgraf, Maria Olímpia Oliveira Rezende

Institute of Chemistry of São Carlos- University of São Paulo. Avenida Trabalhador São Carlense, 400 - CEP: 13566-590, São Carlos-SP, Brasil.

Phone: +551633739959,

E-mail: darlanveggito@hotmail.com, landgraf@iqsc.usp.br, mrezende@iqsc.usp.br



This work is licensed under a [Creative Commons Attribution 4.0 International License](https://creativecommons.org/licenses/by/4.0/).

Abstract

A microwave-assisted extraction (MAE) was optimized for the determination of *p,p'*-DDT in sewage sludge sample. The extraction process was defined by a 2^3 factorial design combined with response surface methodology (RSM). The parameters (temperature, extraction time and the acetone volume in the mixture of solvents ethyl acetate-acetone) were optimized through the response surface methodology. The experimental results shown that the volume of solvent ethyl acetate was significant in the extraction process. Optimal conditions for MAE were temperature of 80 °C, extraction time of 10 minutes and 10 mL of ethyl acetate-acetone (80:20%). This study was accomplishment to evaluate the statistical significance of the parameters temperature, extraction time and acetone volume in the MAE of *p,p'*-DDT in sewage sludge samples. In optimized conditions, recoveries around 98.40% as well as relative standard deviation of 1.21% were achieved.

Keywords: Microwave-assisted extraction, Response surface methodology, Persistent organic pollutants, DDT, domestic sewage sludge

Introduction

Domestic sewage sludge is the residue generated during the treatment of wastewater stations in the Wastewater Treatment Plant that contains nutrients and organic matter that can bring benefits for the soil and consequently serve as an additive in agriculture, increasing soil fertility. However, sewage sludge must be free from contaminants otherwise; it may become a source of contamination. Organochlorine pesticides (OCPs) used in pest control are highly lipophilic and tend to adsorb on solid particles of sewage sludge and effluents [1].

Organochlorine pesticides (DDT, HCB, HCHs etc.) are classified as persistent organic pollutant (POPs) and are of global concern with respect to contamination and environmental toxicity [2]. They are one of the most important environmental contaminants; when released in the environment they may persist or resist to degradation and then remain unchanged for long periods; its resistance to degradation results in universal contamination of water, soil and foods [3]. They are bioaccumulative, i.e., they are resistant to degradation and their wastes accumulate in animals and persist in the environment, rivers, soils, sediments and other living beings, therefore bioaccumulating up along the food chain [4]. This paper focuses on the organochlorine *p,p'*-DDT.

The Stockholm Convention, together with the United Nations Environment Programme (UNEP), has defined alternatives to manage, reduce or eliminate POPs, recognizing that these pollutants have toxic properties, being resistant to degradation, are bioaccumulative and transported by air, water and by migratory species through international boundaries and deposited far from their place of release, where they accumulate in terrestrial and aquatic ecosystems [5].

DDT (1,1,1-trichloro-2,2-bis (4-chlorophenyl) ethane) is still widely used in many types of crops, as well as for the pest control. Its use was banned in many countries because of its negative impact on the animals and their toxic effects on human health via food chain. The Environmental Protection Agency (EPA) from the USA classifies DDT and its breakdown products DDD (1,1-dichloro-2,2-bis (4-chlorophenyl) ethane) and DDE (1,1, dichloro 2,2-bis (4-chlorophenyl) ethylene) as pollutants extremely persistent in the environment [6].

OCPs are considered the second generation of pesticides that took over from the first generation insecticide produced from inorganic compounds (arsenic, lead, copper, and sulfur) in the 1940s. The safety use of these chemical improved the quality of life, ensuring a regular food supply, as well as protection against insect born disease. However, one of the valuable properties of DDT was its persistence, reducing the need for frequent applications. DDT became the prototype OCP from which other more toxic pesticides such as aldrin, dieldrin, lindane, chlordane and heptachlor were developed [7].

Many techniques for OCPs extraction are suitable and effective, but are relatively time-consuming and require large volumes of organic solvents, which are hazardous to human health and cause serious pollution problems. Simple, fast and solvent-saving techniques, such as ultrasound and microwave-assisted extraction are a modern trend in analytical chemistry [8].

Microwave ovens, primarily used for samples digestion, have been also used for the extraction, offering advantages such as increased efficiency, reduced extraction time, low solvent consumption and high level of automation compared to conventional extraction techniques. The principle of this method lies in the fact that

microwave energy is absorbed by extractant, which in turn transfers it to the sample in the form of heat. The partitioning of the analytes from the sample matrix to the extractant depends mainly on the temperature and on the nature of the extractant. Unlike the heating used in the conventional extraction techniques, microwaves heat the entire sample simultaneously [9].

Microwave-assisted extraction of organic pollutants was originally introduced in 1986 by Ganzler [10]. Since then, several applications have been reported the use of microwaves for assisting the extraction of organic and organometallic compounds from liquid and solid matrices [11]. Therefore, factors such as power, temperature, extraction time, solvent, and solvent ratio affect the extraction efficiency [12]. Therefore, an optimization step is required.

The aim of this study was to optimize a methodology for the extraction of *p,p'*-DDT in domestic sewage sludge samples using microwave-assisted extraction followed by gas chromatography-mass spectrometry determination. Only the contamination-free sewage sludge can be safely used as fertilizer in agriculture. Thus, one can provide an adequate environmental destiny for this residue, transforming an unwanted material into a valuable product, contributing for the organic matter recycle.

Materials and Method

Samples

The sewage sludge samples were collected in the Wastewater Treatment Plant of São Carlos city, SP, Brazil, and cooled after the collection. The material sample was prepared from 85 g of sewage sludge in 200 mL of water and used in all experiments. The moisture was determined and considered for *p,p'*-DDT concentration determinations.

Chemicals

Reference material *p,p'*-DDT ($200 \mu\text{g mL}^{-1}$) was obtained from Supelco (Bellefonte, PA, USA). From the reference material, a stock solution of $20 \mu\text{g L}^{-1}$ in ethyl acetate was prepared. Acetone and ethyl acetate were obtained from Panreac and Tedia, respectively.

Chromatographic system

A GC/MS, Shimadzu, GC2010 Plus, equipped with split/splitless injector and a mass spectrophotometric detector was used in the study. The capillary column was a BP-5, 30 m x 0.25 mm i.d. and 1.0 μm film thickness (Agilent). Helium at a constant flow rate of 1 mL min^{-1} was the carrier gas. The injector and detector temperature were 280 °C. The spectrometer operating conditions were: source temperature at 220 °C; interface temperature at 250 °C interface; monitored ions were: 235, 165 (m/z) and retention time for *p,p'*-DDT was 17.18 min. The mass spectrometer was operated in full scan mode for determination of the retention time of the compound and in the SIM mode for the determination of the sample. Gas chromatograph conditions are described in Table 1.

Table 1. Gas chromatograph conditions.

Injection	Splitless (1 min)
Injector temperature	280°C
Column temperature	80°C
Flow	He, 1.0 mL.min ⁻¹
Temperature program	80 °C (1:50 min) + 40 °C min ⁻¹ up to 170 °C + 6.5 °C min ⁻¹ up to 220 °C (4:50 min) + 10 °C min ⁻¹ up to 250 °C (1:00 min)

Microwave-assisted extraction (MAE)

The extraction procedure was performed in a microwave digestion system (SPEEDWAVE FOUR®, with Built-in Non-Contact, Temperature and Pressure Measurement, BERGHOF, Germany) with a capacity of 12 vessels of 60 mL and 1450 W maximum power. To the optimization tests, 10.00 mL of sample (9.00 mL of the sewage sludge solution + 1.00 mL of the DDT sock solution) plus 10.00 mL of the mixture of solvents ethyl acetate-acetone was used. The extraction temperature ranged from 80 to 120 °C, acetone volume in solvent ranged from 20 to 80% (in volume), the irradiation times evaluates were 10 and 30 minutes. The magnetron power output of the microwave unit was set at 80% (1160 W). The ramp time (8 min) was previously defined, evaluating the time necessary for the equipment to reach the programmed maximum temperature (120 °C), using the lowest possible power.

After the extraction step, the vials were opened after cooling until room temperature. Then, the material was filtered in 45 µm filter paper, concentrated under vacuum in rotary evaporator (FIZATOM) at 80 °C until dryness, reconstituted with 10.00 mL of ethyl acetate and placed in a vial for the chromatographic determination. For tests with blanks, the above procedure was followed using one sample without addition of the reference material. Table 2 shows the MAE operational conditions.

Table 2. MAE operational conditions

Magnetron power	80% (1160 W)
Ramp*	8 min
Extraction temperature	80-120°C
Extraction time	10-30 min
Solvent volume	10 mL

Sample volume	10 mL
Maximum vessel pressure	35 bar

*time to reach settings

Experimental design and optimization of microwave-assisted extraction

A 2^3 factorial design comprising 16 experiments in duplicate was chosen for the MAE optimization. For statistical calculations (to create experimental design and to analyze the experimental data), the software Origin 8.0 was used. All experimental runs were defined according to preliminary experiment and programming conditions. The settings of the three factors are given in Table 3.

Table 3. Factors and their settings for 2^3 experimental design for MAE

Factor	Minimum	Maximum
Temperature (°C)	80	120
Extraction time (min)	10	30
Acetone in ethyl acetate mixture (vol.%)	20	80

A two-way interaction linear model was adjusted to the experimental data based on the equation 1:

$$\hat{y} = \beta_0 + \beta_1x_1 + \beta_2x_2 + \beta_3x_3 + \beta_{12}x_1x_2 + \beta_{13}x_1x_3 + \beta_{23}x_2x_3 \quad (1)$$

where \hat{y} is the predicted value (area), β 's are the model coefficients, and x_1, x_2, x_3 the encoded factors: temperature (x_1 , 80-120 °C), extraction time (x_2 , 10-30 min) and acetone volume (x_3 , 20-80%).

Results and Discussion

Extraction

The solvent chosen for extracting was the mixture ethyl acetate-acetone. The use of microwaves energy in the extraction process generates heating of solvent, and consequently of the sample which can suffer degradation of some compounds in different ways due exposure to high temperatures and pressure inside the extraction vessels [3]. To achieve the best extraction conditions it was used the response surface methodology. The actual and encoded levels of the independent variables (temperature, extraction time, and acetone volume) for each experiment as well as the response-variable (area) obtained are shown in Table 4.

Table 4. Experimental design 2³: actual and encoded levels of the factors and observed responses.

Factors			Average responses*
(1) Temperature (°C)	(2) Extraction time (min)	(3) Acetone (vol. %)	
80 (-1)	10 (-1)	20 (-1)	4735.385
120 (+1)	10 (-1)	20 (-1)	3626.993
80 (-1)	30 (+1)	20 (-1)	3341.099
120 (+1)	30 (+1)	20 (-1)	2239.929
80 (-1)	10 (-1)	80 (+1)	1547.099
120 (+1)	10 (-1)	80 (+1)	465.6435
80 (-1)	30 (+1)	80 (+1)	2056.543
120 (+1)	30 (+1)	80 (+1)	2325.17

*(n=3)

Estimated model

A polynomial model was adjusted to the experimental data (Equation 2). The corresponding ANOVA (Analysis of Variance) data are shown in Table 5.

$$\hat{y} = 2542 - 378x_1 - 51x_2 - 944x_3 + 42,41578x_1x_2 + 43,64790x_1x_3 + 160,9475x_2x_3 \quad (2)$$

Table 5. Analysis of variance (ANOVA) for the experimental results.

Source	Sum of squares	Degrees of freedom	Mean of square	F-value	P-value
Model*	2.09206E7	3	6.97354E6	22.50142	3.23513E-5
X ₁	2.28371E6	1	2.28371E6	1.43013	0.25161
X ₂	42514.109	1	42514.109	0.02420	0.87860
X ₃	1.42467E7	1	1.42467E7	19.1912	6.27621E-4
X ₁ X ₂	460569.22	1	460569.22	0.25292	0.62413
X ₁ X ₃	487715.77	1	487715.77	0.76790	0.39807

X_2X_3	6.63145E6	1	6.63145E6	21.3976	5.84362E-4
Error	3.71899E6	12	309915.70		
Cor. total	2.46396E7	15			
	$R^2 = 0.9990$	$R^2_{\text{adjusted}} = 0.9973$			

* X_1 = temperature, X_2 = extraction time, X_3 = acetone volume

High F -value associated with low P -value ($P < 0.05$) demonstrated that the generated model was statistically significant [13]. P -values are used to confirm the significance of each coefficient, i.e., in the low P -value is the greatest effect [14]. The total determination coefficient ($R^2 = 0.9990$) indicated that the model could explain 99.90% of the variation in p,p' -DDT extraction.

According to analysis of variance, the F -values (Table 4), the parameter with the greatest effect is the factor interactions extraction time and acetone volume in the mixture of solvents (X_2X_3), followed by factor X_3 (acetone volume).

Response surface plot analysis

Response surface graphics are very useful to estimate the effects of two factors on the response, as well as the identification of optimal values for maximum response [13]. Graphs were plotted using the z -axis (response) against two of the independent variables (temperature, extraction time, and acetone volume), maintaining a third one at a fixed value. Figures 1, 2 and 3 show the main interactions of the process: (temperature x extraction time, temperature x solvent, and extraction time x solvent).

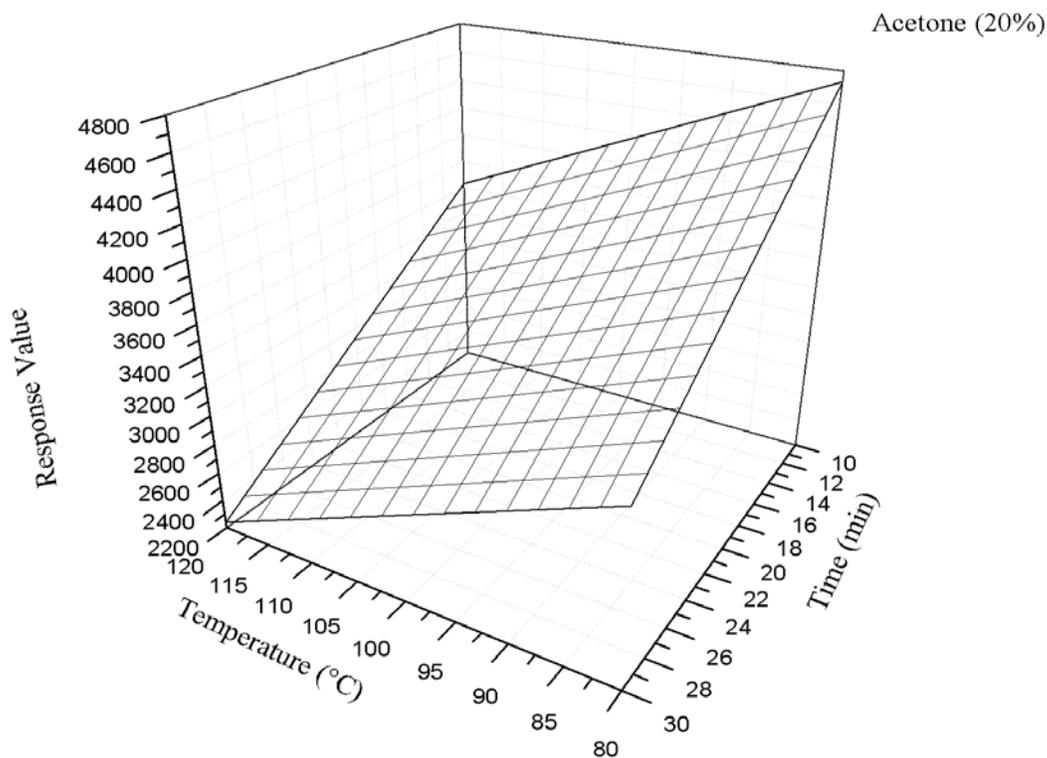


Figure 1. Response surface. Temperature x extraction time.

Figure 1 shows the interaction between the temperature and the extraction time in the response. By decreasing the temperature from 120 to 80°C and decreasing the extraction time from 30 to 10 minutes, the responses is improved. The extraction equilibrium was achieved within de 10 min. Is probable that the contact surface area between the analyte and the extraction solvent was very large. Thus, extraction equilibrium can be achieved in short time and the phase transfer of the target analyte was fast. The best responses are achieved at smaller temperature (80°C) and smaller extraction time (10 min). The factor interactions temperature and time of extraction are critical factors in the extraction of analytes, i.e, are related to the maximum extraction of analytes and their sensitivity to degradation.

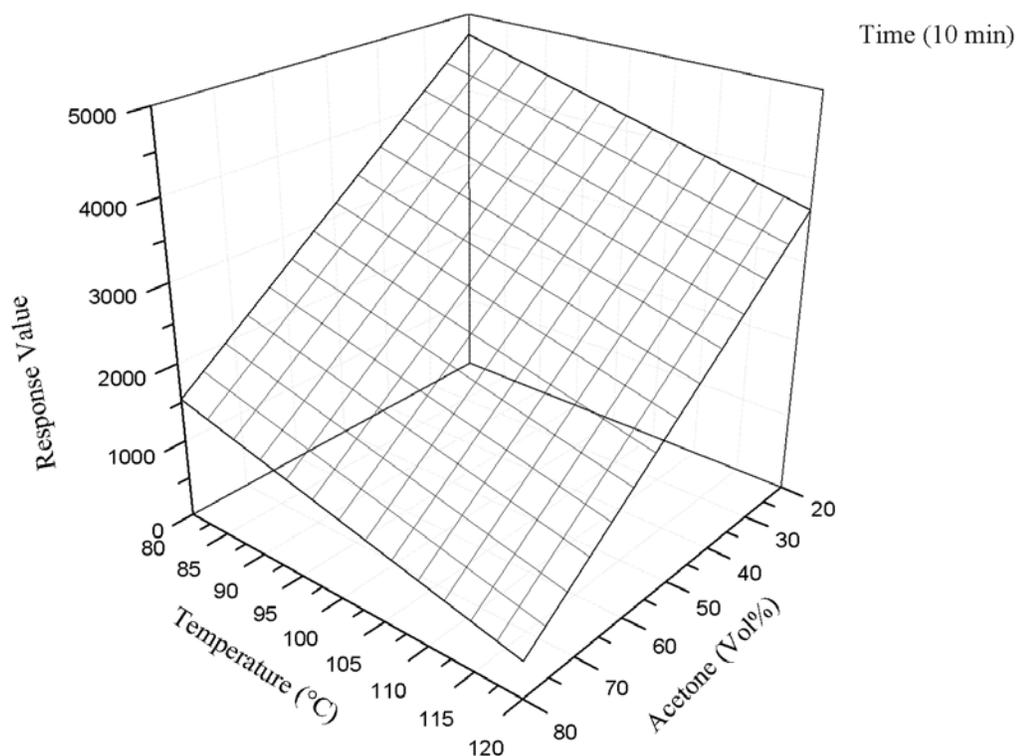


Figure 2. Response surface. Temperature x Acetone (Vol%).

Figure 2 shows the interaction between the temperature and acetone volume. Decreasing the temperature from 120 to 80 °C and using 20% of acetone improves the efficiency of extraction. Although both solvents had low dielectric loss factor ($\tan \delta < 0.1$), the improvement in the response with the decrease of the percentage of acetone in the mixture indicates the effectiveness of the ethyl acetate solvent due to its polarity and low volatility.

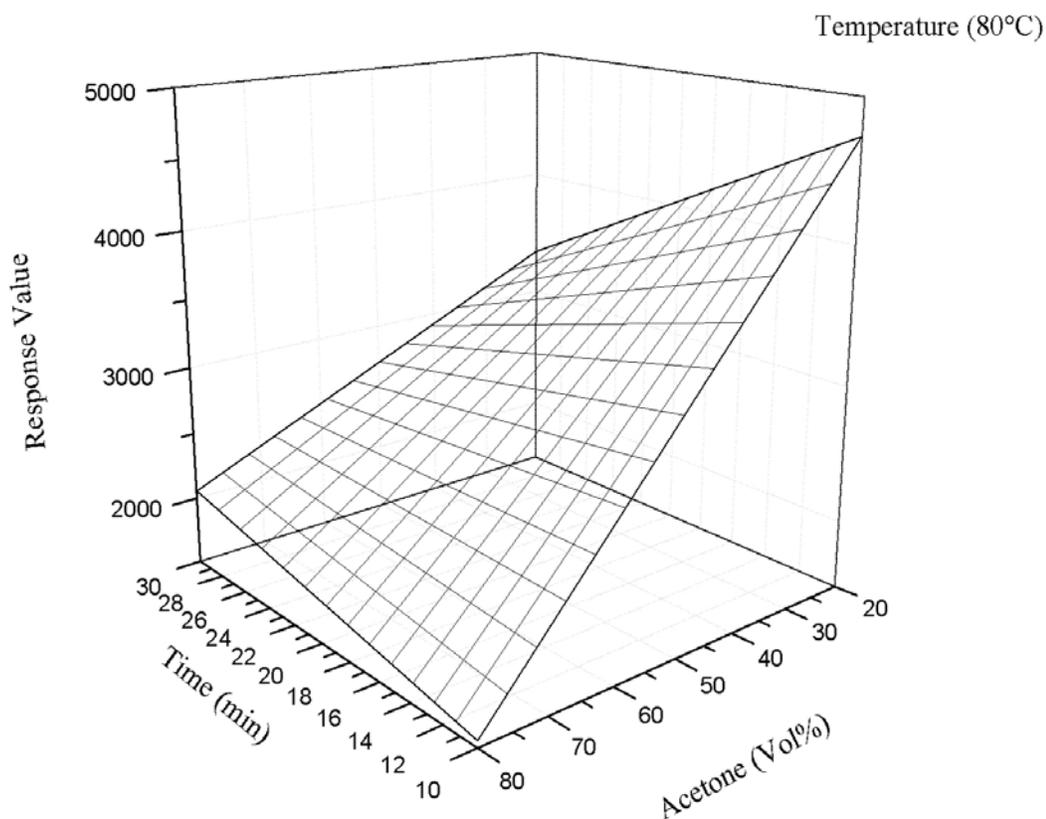


Figure 3. Response surface. Extraction time x Acetone (Vol%).

Figure 3 shows that best responses are achieved when the extraction time decreases from 30 to 10 min and a smaller volume of acetone is used, i.e, the factors interactions indicates that a lowest extraction time, reduces the risk of degradation of the target compounds, and the lower volume of acetone promotes the extraction by the ethyl acetate solvent.

Recoveries assay: yield of extraction

After optimization of the methodology, the yield of extraction was conducted using the parameters defined as the best response in the optimization of microwave assisted extraction, ie, temperature of 80°C; mixture ethyl acetate: acetone (80:20%), and extraction time of 10 minutes. The recovery assays experiments were performed by adding 1.00 mL of the stock solution of *p,p'*-DDT directly to the extraction vessels, so the final concentration in 20 mL of the solution was equal to 1.00 $\mu\text{g mL}^{-1}$. After the extraction, the sample was determined according to the previously described procedure. In the analysis of blanks, no chromatographic peaks related to *p,p'*-DDT were observed.

Table 6 shows the data of the linear regression, coefficient of determination, recovery and relative standard deviation of the *p,p'*-DDT.

Table 6. Linear regression, coefficient of determination, recoveries and relative standard deviation for *p,p'*-DDT.

Compound	Linear regression	R ²	Average recovery* (%)	R.S.D (%)
<i>p,p'</i> - DDT	y= 42.1294x – 1180.484	0.9805	98.40 ± 0.71	1.21

* Means ± standard deviation (triplicate) and relative standard deviation (R.S.D).

Considering the significance of the factor temperature as well as recovery levels achieved, the microwave-assisted extraction was satisfactory, whereas the lowest temperature and shortest extraction time were sufficient to obtain the best responses

In agroecological question, the sewage sludge can be used as fertilizer due to have large load of nutrients and organic matter derived from the previous steps your training. However, the presence of pesticides such as DDT and other potentiate the toxicity of this waste. Thus, this paper describes an alternative method for the extraction of *p,p'*-DDT in sewage sludge from the perspective of green analytical chemistry, as well as a means to monitoring of contamination for that pesticide in other matrices.

Conclusion

MAE is an advantageous technique for extraction of the organochlorine compound *p,p'*-DDT in sewage sludge samples. As could be shown, the extraction solvent ethyl acetate could be used in all extraction tests. Furthermore, the recoveries obtained with the described procedure were satisfactory.

In general, the factor interactions between acetone volume (20%) and extraction time (10 min) were the most significant in the process. Analyzing the extraction solvent, the lower acetone volume in the mixture shows a positive effect. The optimal conditions were reached at a maximum temperature of 80° C; solvent mixture ethyl acetate-acetone (80: 20%) and extraction time of 10 minutes.

Acknowledgement

The authors would like to acknowledge to FAPEMA (Maranhão Foundation for the Protection of Research and Scientific and Technological Development) under Grant BD-01741/12, CNPq (National Council for Scientific and Technological Development) under Grant 306715/2013-9, FAPESP (São Paulo Research Foundation) under Grant 2013/13093-7 and NAP 2012-CiTecBio, Provost for Research, University of São Paulo.

References

- [1] Sánchez-Brunete, C.; Miguel, E.; Tadeo, J. L. Determination of organochlorine pesticides in sewage sludge by matrix solid-phase dispersion and gas chromatography-mass spectrometry. *Talanta*, 74 (2008) 1211-1217. DOI: 10.1016/j.talanta.2007.08.025
- [2] Tolosa, I.; Mesa-Albernas, M.; Alonso-Hernandez, C. M. Organochlorine contamination (PCBs, DDTs, HCB, HCHs) in sediments from Cienfuegos bay, Cuba. *Marine Pollution Bulletin*, 60 (2010) 1619-1624. DOI: 10.1016/j.marpolbul.2010.06.008
- [3] Barriada –Pereira, M.; Concha-Grana, E.; González-Castro, M. J.; Muniategui-Lorenzo, S.; López-Mahía, P.; Prada-Rodríguez, D.; Fernández-Fernández, E. Microwave-assisted extraction versus Soxhlet extraction in the analysis of 21 organochlorine pesticides in plants. *Journal of Chromatography A*, 1008 (2003) 115-122. [http://dx.doi.org/10.1016/S0021-9673\(03\)01061-6](http://dx.doi.org/10.1016/S0021-9673(03)01061-6)
- [4] Li, H. P.; Li, G. C.; Jen, J. F. Determination of organochlorine pesticides in water using microwave assisted headspace solid-phase microextraction and gas chromatography. *Journal of Chromatography A*, 1012 (2003) 129-137. DOI: 10.1016/S0021-9673(03)00916-6
- [5] Mörner, J.; R.Bos & M. Fredrix. Reducing and eliminating the use of Persistent Organic Pesticides - Guidance on alternative for sustainable pest and vector management. Inter-Organization Programme for the Sound Management of Chemicals. Geneva, 2002.
- [6] Xia, C.; Ma, X.; Liu, S.; Fan, P. Studies on Remediation of DDT-Contaminated Soil and Dechlorination of DDT. *Procedia Environmental Sciences*, 16 (2012) 289-292.
- [7] Clarke, B. O.; Porter, N. A.; Marriott, P. J.; Blackbeard, J. R. Investigating the levels and trends of organochlorine pesticides and polychlorinated biphenyl in sewage sludge. *Environment International*, 36 (2010) 323-329. DOI:10.1016/j.envint.2010.01.004
- [8] Mao, X.; Wan, Y.; Yan, A.; Shen, M.; Wei, Y. Simultaneous determination of organophosphorus, organochlorine, pyrethroid and carbamate pesticides in *Radix astragali* by microwave-assisted extraction/dispersive-solid phase extraction coupled with GC-MS. *Talanta*, 97 (2012) 131-141. DOI: 10.1016/j.talanta.2012.04.007
- [9] Papadakis, E. N.; Vrizas, Z.; Papadopoulou-Mourkidou, E. Rapid method for the determination of 16 organochlorine pesticides in sesame seeds by microwave-assisted extraction and analysis of extracts by gas chromatography-mass spectrometry. *Journal of Chromatography A*, 1127 (2006) 6-11. DOI: 10.1016/j.chroma.2006.06.010
- [10] Ganzler, K.; Salgo, A.; Valko, K. *Journal of Chromatography*. 371 (1986) 299.
- [11] Gfrerer, M. & Lankmayr, E. Screening optimization and validation of microwave extraction for the determination of persistent organochloride pesticides. *Analytica Chimica Acta*, 533 (2005) 203-211. DOI: 10.1016/j.aca.2004.11.016

- [12] Hayat, K.; Hussain, S.; Abbas, S.; Farooq, U.; Ding, B.; Xia, S.; Jia, C.; Zhang, X.; Xia, W. Optimized microwave-assisted extraction of phenolic acids from citrus mandarin peels and evaluation of antioxidant activity *in vitro*. *Separation and Purification Technology*, 70 (2009) 63-70. DOI: 10.1016/j.seppur.2009.08.012
- [13] Karabegovic, I. T.; Stojicevic, S. S.; Velickovic, D. T.; Nikolic, N. C.; Lazic, M. L. Optimization of microwave-assisted extraction and characterization of phenolic compounds in cherry laurel (*Prunus laurocerasus*) leaves. *Separation and Purification Technology*, 120 (2013) 429-436. DOI: 10.1016/j.seppur.2013.10.021
- [13] Zhao, W.; Yu, Z.; Liu, J.; Yu, Y.; Yin, Y.; Lin, S.; Chen, F. Optimized extraction of polysaccharides from corn silk by pulsed electric field and response surface quadratic design. *Journal of the Science of Food and Agriculture*, 91 (2011) 2201-2209. DOI: 10.1002/jsfa.4440