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Polychlorinated Biphenyls in Groundwater of Grombalia: Optimization and Validation of Analytical Procedures Using Gas Chromatography with Electron Capture Detector

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Abstract

Now-a-days, the management of water resources is one of the main global challenges, both at the level of agricultural and industrial activities as well as direct consumption and poses various problems of both qualitative and quantitative order. In the entire world, groundwater suffers from various sources of contamination principally due to overusing of chemical fertilizer in the agricultural sector. The contamination of groundwater by organochlorines and more precisely by polychlorinated biphenyls (PCBs) is a problem of global order. As we know that Tunisia is based on agriculture work like citrus, olive, wheat and cereal, the study of water pollution is a topical subject that remains unclear. Polychlorinated Biphenyls (PCBs) are one class of persistent organic pollutants. PCBs in recent decades have attracted the attention of scientific and policy maker communities due to their persistence, their high capacity to bioaccumulation in the food chain and their toxic properties. This work is aimed to develop an efficient method for the analysis of targeted PCBs in groundwater sample taken from the region of Grombalia. Optimization of PCBs extraction was performed with applied the experimental design of Dohlert with two factors, solvent and number of extractions. Analysis of PCBs was performed with gas chromatography coupled with electron capture detector (GC-ECD) with an optimization of temperature program. Results shows that the optimal temperature program was obtained with a starting temperature of 160°C up to 280°C during 10 min with a rate of 4°C min^{-1} and the optimal condition of extraction was obtained with a mixture of hexane/ether (75/25%) and a three time extractions. The optimized method has been applied to the analysis of the PCBs in nine groundwater samples collected from the Grombalia city. Results indicate that PCBs concentrations varied between 5.2 μgL^{-1} and 169 μgL^{-1}. However, the maximum acceptable concentration (MAC) in drinking and surface water recommended by EPA is 0.5 μgL^{-1} with a detection limit (LD) ranged between 0.05 to 1.9 µg L^{-1}.

Keywords: Polychlorinated biphenyl • Liquid-liquid extraction • Gas chromatography with ECD • Doehlert experimental design • Groundwater of Grambalia

Introduction

Since 1930, polychlorinated biphenyls (PCBs) are only industrial products and were applied in various field of activity. This one was divided to open and close application such, respectively, capacitors stabilizing additives in PVC coatings, plasticizers in paints and cements, pesticide extender and hydraulic fluids, transformer and cutting oil, paints, etc., [1]. Since 22 May 2001, this pollutant has been cited in Stockholm convention like persistent organic pollutants (POPs) [2].

Seen in the biphenyl structure, two linked benzene rings with 1 to 10 chlorine atoms, we find in total 209 congeners of PCBs with high resistance to degradation, high persistence, and non-solubility in water, toxicity and bioaccumulation properties [3]. Due to these one, PCBs exposures ensue big health problems like cancer, neurologic and immune-toxic effects. Depending on the degree of congeneration, the half-life of PCB varied from 10 days to decades [4]. PCBs can be bio-accumulate in fish [5], meats and dairy products [6,7] also in bottom sediments and aquatic environments at owing to their low solubility in water and their high octanol-water [2,8]. To resume, food consumption has been and still the biggest exposure source of population [9]. PCBs analysis is done with chromatographic techniques and more precisely the gas chromatography (GC) [10] and high performance liquid chromatography (HPLC) [11]. In the literature, gas chromatography coupled with electron capture detector (ECD) is frequently used for the trace analysis of environmental pollutants, such as PCBs and pesticides, due to its high sensitivity to electronegative elements [12]. ECD detector has proven its efficiency in the analysis of PCBs in surface and groundwater [13,14].

In recent works, many researches treated the problematic of PCBs presence in the environment from different ways: as a chemo stratigraphic marker of the Anthropocene [15], PCBs levels in surface sediments and drinking water [16], searching new technologies to cleanup and treatment of water contaminated with persistent organic pollutants [17,18] and the development of selective extraction method for the quantification of 84 polychlorinated biphenyls and organochlorine pesticides in shellfish sample [19] but we can notice that studies about the PBCs contamination in the ground water still not developed or not token in consideration may be consequent to PCBs proprieties. That's why, in the present study, an optimization of the method of PCBs analysis in groundwater of Tunisia was developed using an experimental design and gas chromatography coupled with electron capture detector (ECD).

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Materials and Methods

Study area and sample collection

Nine water samples were collected from Grambalia city in the governorate of Nabeul from different wells along a current line about 12 Km extended from Bou Argoub up to Sebkha Soliman, as shown in Figure 1. This area includes several urban and industrial agglomerations such as Soliman, Bou Argoub, Grombalia and Menzel Bouzelfa with several industrial areas (Grombalia, Bou Argoub and Soliman). Water was collected using pre-cleaned amber glass bottles and was transported to the laboratory immediately after sampling. The samples were stored in the refrigerator at 4°C until analysis. The sampling campaigns were conducted twice a year in April 2014 and August 2014, respectively.

Reagents

All chemicals and reagents were of analytical grade and of the highest purity possible. Hexane, dichloromethane and ether were obtained from Scharlau. Florisil used in the cleaning of the extract were suffered by Fluka. Different concentrations of standard mixture containing seven PCBs (CB-28. 52. 101. 153. 138. 180 and 209) provided by Merch; Germany.

Analytical procedures

The optimization of the analysis method was realized with real sample collected from well (P9 in the line current).

Optimization of sample extraction using experimental design methodology: According to the bibliographical study, there various types and model of experimental design and all of them were used in the optimization of experimental response and to make a clear process to planning the experimental methodology [20].

In order to develop an extraction procedure of PCBs from the groundwater matrix, an experimental design of Doehlert created by NEMRODW Software was usedto represent the responses of the two factors in the all experimental studied field [21].

Doehlert experimental design is useful specifically in the optimization of experimental method since due to its presentation of uniform distribution of experimental points in the studied area [22].

To evaluate the influence of operating parameters on the extraction recovery of PCBs, two parameters were chosen like variables: organic solvent or mixture of solvent volume used for extraction (K1) and number of extraction repetition (K2). A factorial design 2k was carried out to determine the influence of these two selected factors and to study their interaction to find the optimum response (Y). Each variable (k) can take two levels minimum and maximum respectively associated to (-1) and (+1) values. The response(Y) associated to our design given by the software under the form of linear polynomial equation.

Two factors were considered like parameters which can impact the extraction recovery (Y). The experimental values corresponding to the two levels of each factor are listed in Table 1.

Inorder to compare the effects of the different factors in the experimental field, concerned coded variables were used. The factors are given in the form of coded variables (Xi) without units inorder to permit comparison of factors of different natures. The response (Y) can be described by a second order model for predicting there sponse in all experimental regions from the following equation:

$$Y=b0+b1*X1+b2*X2+b11*(X1*X1)+b22*(X2*X2)+b12*(X1*X2)$$

Groundwater samples (1 L) were extracted using liquid–liquid extraction (LLE) by 100 ml of organic solvent, using experimental design presented in Tables 2 and 3.

The extracts were then concentrated, before the purification step, to a volume of 5 mlbyrotary evaporator and then to 1 ml under nitrogen-flow.

Optimization of purification and separation: A glass column was filled with florisil (prebaked at 300°C for 4 h) with the bottoms and plugged with cleaned glass wool. In the top of the column 2 g of anhydrous sodium sulphate was added to eliminate eventual traces of water. Targeted compounds were recovered by successive elution with 70 ml of hexane as fraction one (F1). Then, with 45 ml of a mixture of hexane and dichloromethane (70:30 V/V) as fraction 2 (F2). The last elution with 60 ml of dichloromethane as fraction (F3).

Optimization of gas chromatography analysis: The samples were

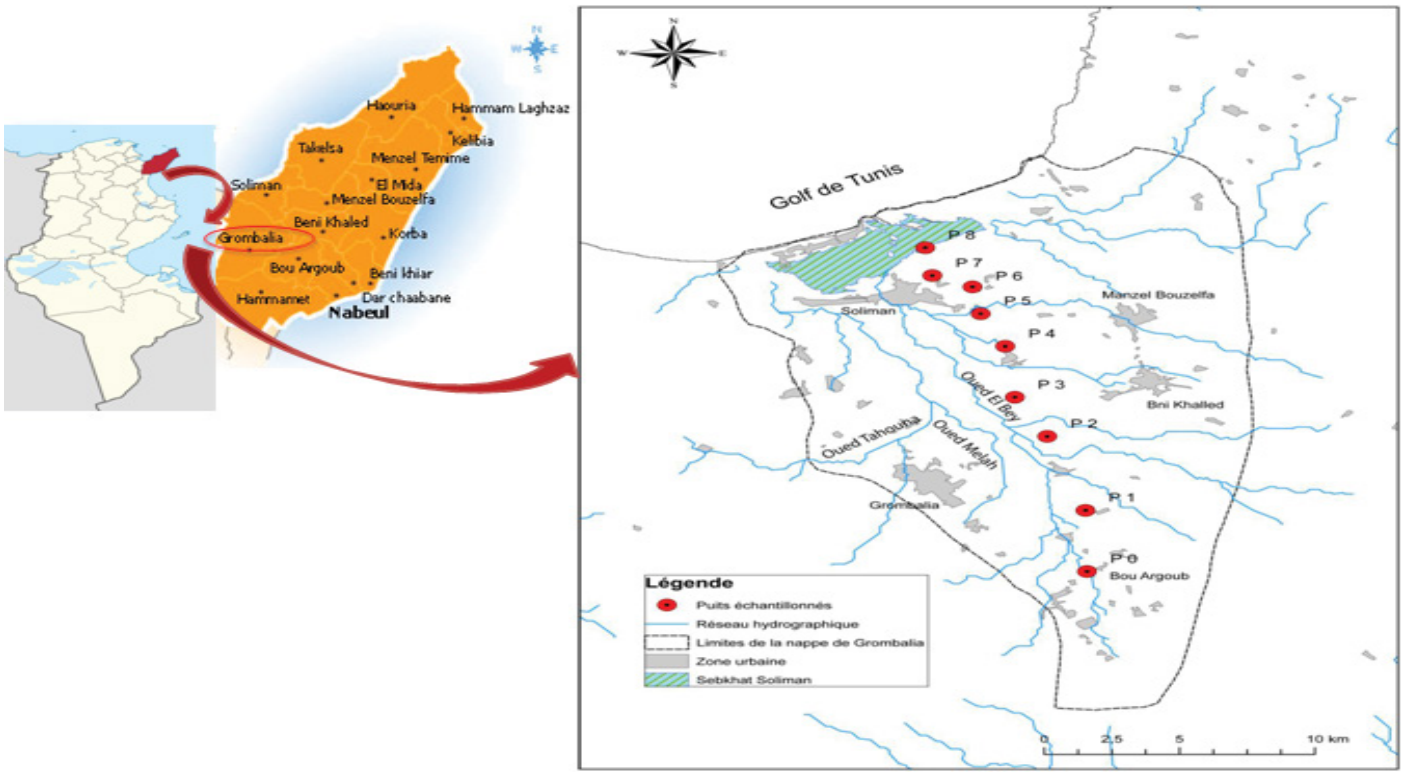

Figure 1. Map representative of the line current water samples studied.

Table 1. Investigated variables and their levels studied in the 2^2 factorial design.

Coded variables (Xi)	Factors(Ki)	Unit	Experimental field	
			Minimum value (-1)	Maximum value (+1)
X1	Percentage extraction volume solvent 1 and 2	ml	0	100
X2	Extraction number	-	1	3

Table 2. Doehlert matrix experiments for the extraction of PCBs with DCM/hexane (plan 1).

Experiences	% DCM/Hexane	Extraction number
1	100	2
2	0	2
3	75	3
4	25	1
5	75	1
6	25	3
7	50	2
8	50	2
9	50	2

Table 3. Doehlert matrix experiments for the extraction of PCBs with ether/hexane (plan 1').

Experiences	% Ether/Hexane	Extraction number
1'	100	2
2'	0	2
3'	75	3
4'	25	1
5'	75	1
6'	25	3
7'	50	2
8'	50	2
9	50	2

analyzed by gas chromatography equipped with a Ni 63 electron capture detector (GC-ECD) and fused silica capillary column (15 m.0. 25 i.d. 0.25 µm). Helium was used as the carrier gas with a flow rate of 1 ml min^{-1}. The injector temperature was maintained at 250°C. Injection volumes were 1 µL in the splitless mode. The detector was maintained at 300°C. The column temperature was initially held at 160°C, ramped to 240°C at a rate of 4°C min^{-1}; then ramped to 280°C at a rate of 10°C min^{-1} and held for 10 min.

Results and Discussion

Optimization of extraction method

The experimental results of the liquid-liquid extraction of PCBs according to the two experimental design of Doehlert are shown in Table 4.

Representation of optimum result by Nemrodw

Liquid-liquid extraction with DCM/Hexane

Equation: Y=0.01437-0.00733*X1+0.00693*X2-0.00137*(X1*X1)-0.00603*(X2*X2)+0.00346*(X1*X2)

Variation of the response: PCBs concentration in the plane: % DCM/hexane, number of extraction (Figure 2).

Liquid-liquid extraction with Ether/Hexane

Equation: Y=0.05657-0.01950*X1+0.02512*X2-0.03957*(X1*X1)-0.01590*(X2*X2)-0.03753*(X1*X2)

Variation of the response: PCBs concentration in the plane: % Ether/hexane, number of extraction (Figure 3).

The comparison of the results (Table 4 and Graphic Study) shows that the plane (1') presents the best concentration of PCBs extracted; this can be explained by the good PCBs affinity to the mixture of organic solvent ether/Hexane and the bad one to the DCM/Hexane. Also, with an extraction number equal to 3, the recovered concentration is better than 1 or 2 (such as (1 '-4) and (1' -6), (1-5) and (1-3) ...).

Table 4. Experimental results.

Experiences	Sum concentrations of 7 PCBs (ppm)
Plan 1: DCM/Hexane	
(1-1)	0.0001
(1-2)	0.0263
(1-3)	0.0193
(1-4)	0.0029
(1-5)	0.0038
(1-6)	0.0115
(1-7)	0.0137
(1-8)	0.0150
(1-9)	0.0141
Plan 1': Ether/Hexane	
(1'-1)	0.0081
(1'-2)	0.0259
(1'-3)	0.0201
(1'-4)	0.0174
(1'-5)	0.0093
(1'-6)	**0.0925**
(1'-7)	0.0583
(1'-8)	0.0626
(1'-9)	0.0627

The majority of PCBs studied have a high degree of substitution (209,

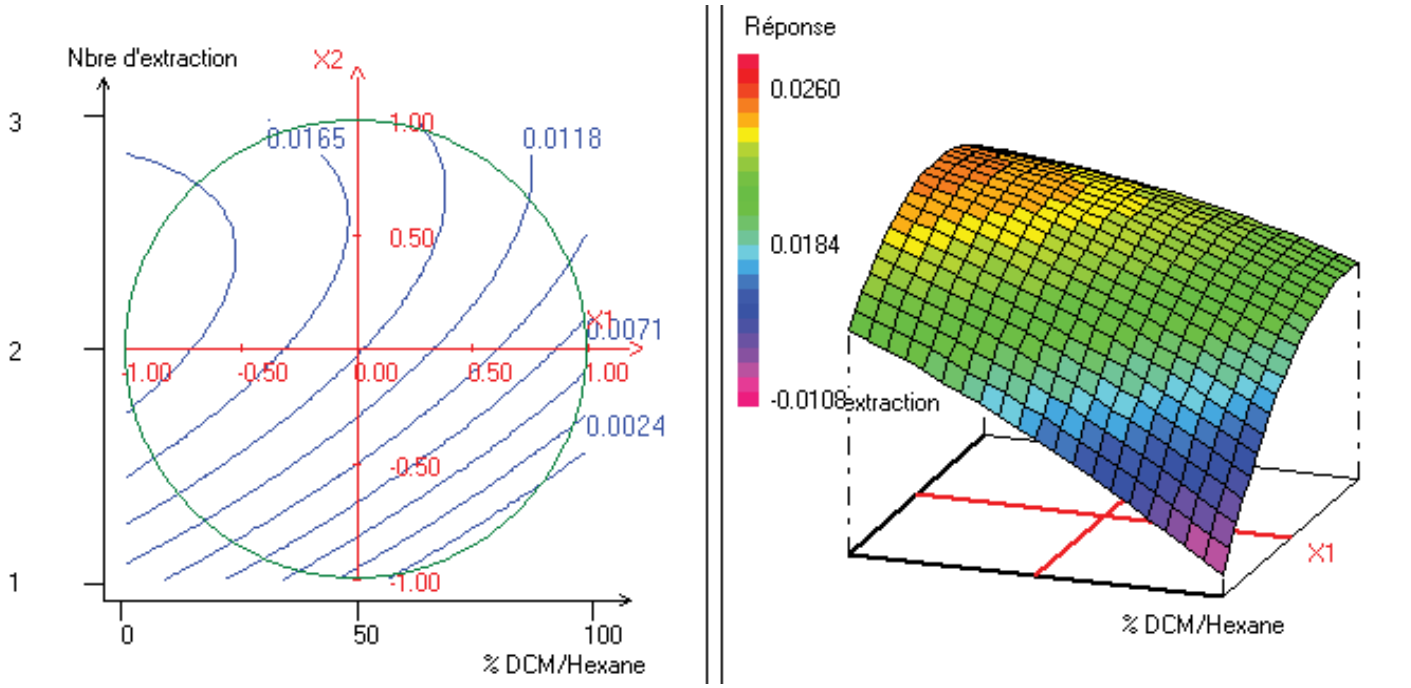

Figure 2. 2D and 3D graphic study plan 1.

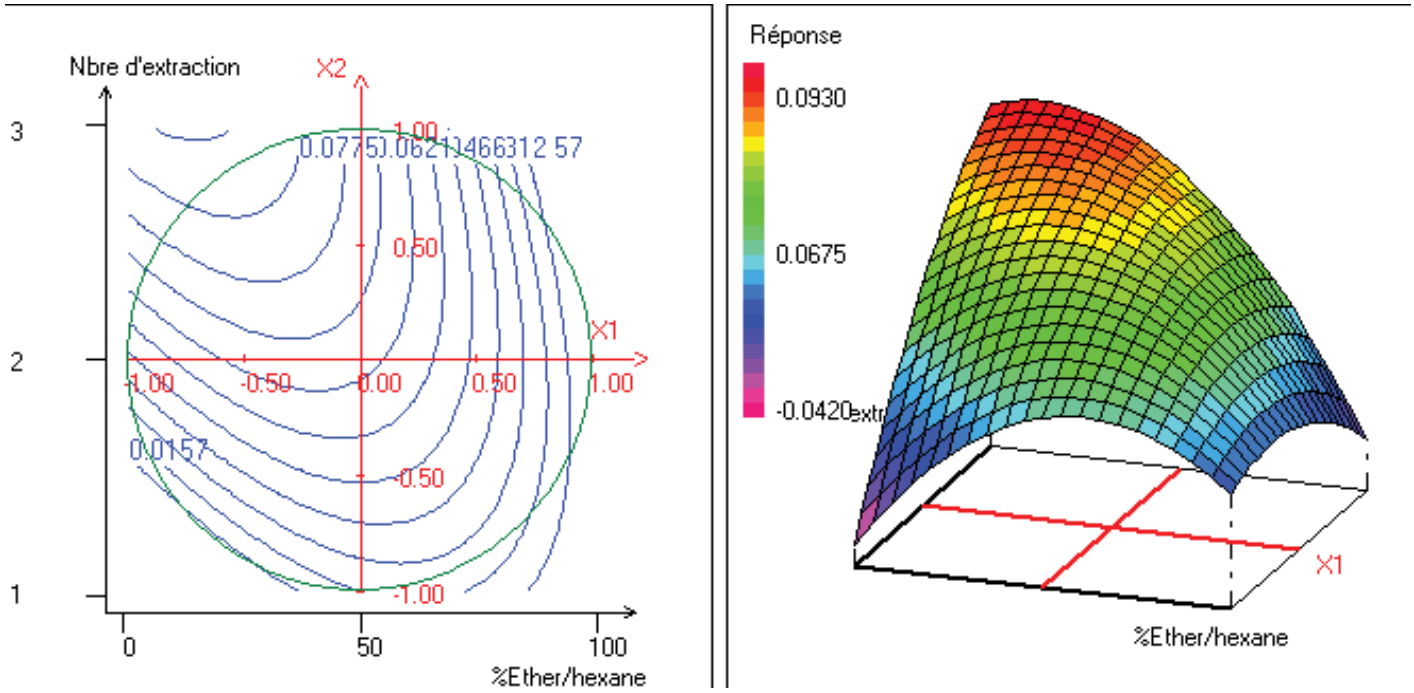

Figure 3. 2D and 3D graphic study plan 1'.

153, 180, 138, 101), for that, they have an affinity to be extracted by hexane because is non-polar solvent and the least substituted ones (28, 52) have to be extracted with ether (shows the concentrations of each PCB congener).

Since, in the experience 1'-6 in which we have a mixture of hexane/ether (75/25%) with three time extractions, the 7 PCBs were detected with a total concentration of 0.092 ppm PCBs. In addition, its chromatogram shows a good peak separation and retention of the seven PCBs with maximum peaks for each one (Figure 4).

Optimization of purification step and separation

Analysis of different fractions shows that PCBs were found with a percentage of 97% in the fraction 1 following by 3% in the fraction 2. For this reason and for the rest of this work, fraction F1 was considered for the step of purification and separation [23].

Limit of linearity and quantification

Working standard solutions of PCBs were run at the beginning of sample analysis to determine peak response and evaluate peak resolution. The limit of detection (LD) and quantification (LQ) was determined as (Table 5):

LQ=3 × signal-to-noise

LQ=10 × signal-to-noise

Respectively, LDs and LQs for PCBs ranged from 0.05 µg L^{-1} to 1.9 µg L^{-1} and 0.16 µg L^{-1} to 6.4 µg L^{-1}. The values obtained were comparable to those found in the literature [16]. The limits obtained in the analysis of PCBs, PAHs and pesticides [24] by GC-MS varied between 0.4 µg.L^{-1}-100 µg.L^{-1} this shows the value of working with ECD detector and its specific character for chlorinated compounds.

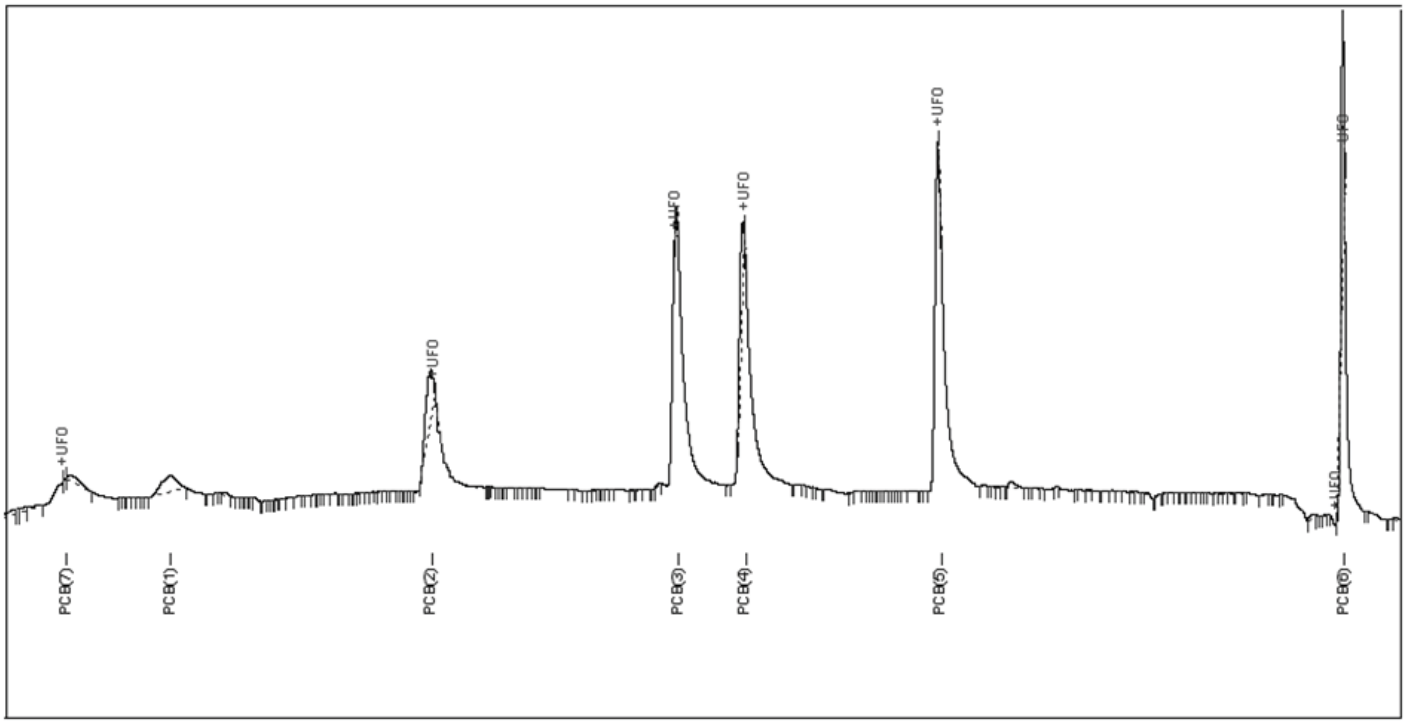

Figure 4. Chromatogram of the optimized extraction method (experience 1.6').

Table 5. LOQ and LOD.

PCBs	LD (ppm)	LQ (ppm)
CB 28	0.0012	0.0041
CB 52	0.0013	0.0042
CB 101	0.0017	0.0058
CB 153	0.0019	0.0064
CB 138	0.0010	0.0035
CB 180	0.0011	0.0038
CB 209	0.00005	0.00016

Limit of linearity

Calculating limits of linearity showed that the method is linear. Since, correlation coefficients range from 0.995 to, 0.999 showing that our method is linear. Limits of linearity are variable for each studied PCBs individually as their degrees of substitution (Table 6).

Recovery test

To determine the efficiency of extraction, we calculated the recoverability testing by adding a known quantity of PCBs to the water sample. The accuracy in terms of percent recovery (Table 7) was calculated with the following equation:

Recovery(%)=((peak area of pre-extraction spike × 100)/(peak area of post extraction spike))

The distribution of PCBs along the studied current line of Grambalia

To test the validated method and to get an idea about the level of groundwater contamination by PCBs, the method optimized was applied to water samples from different wells located in Grombalia Nabeul governorate.

Each sample was analyzed by the chosen method. Therefore, each sample underwent analysis to determine its physical-chemicals qualities (Table 8).

The concentrations of PCBs detected in the groundwater range from 0.0052 ppm to 0.169 ppm. Comparing the distribution of PCBs concentrations, we considerate that the influential parameters in the penetration of PCBs in the water table are the piezo metric level and the charge of material suspension. In fact, when the groundwater level is low, the charge of material in suspension increase. For that, adsorption of PCBs by particles in suspension was accelerated and frequently leads to a relative enrichment of water by low substituted PCBs [8] which proves the high concentration (0.169 ppm) found in P6 which the congeners the least substituted (PCBs 28) having the higher concentration of 0.16 ppm (results of PCBs congeners concentrations in each well, shown in Figure 5).

Table 6. Correlation coefficient and limits of linearity.

PCBs	Limite de Linearite (mg L^{-1})	Coefficient de Correlation (R^2)
CB 28	0.39	0.999
CB 52	0.4	0.999
CB 101	0.42	0.997
CB 153	0.46	0.995
CB 138	0.44	0.998
CB 180	0.5	0.999
CB 209	0.5	0.999

According to study made by Mouna Ncibi about the distributions of organochlorine pesticides and polychlorinated biphenyl in surface water from Lagoon in Tunisia the average concentrations of PCBs were 3 and 10.4 ng L^{-1} [25] and following the work of Samia Khadhar PCBs concentrations in the ground water of the Cap Bon-Tunisia range between 0.0052 and 0.196 mg/L [26]. These results are comparable to ours and make us think to focus on the degree of toxicity that threatens our groundwater resources by these toxic and persistent compounds.

Table 7. Recovery percentage for each PCB analyzed.

PCBs	Concentration in the Raw Sample (1'-6) (mg.L^{-1})	Standard Concentration Added (± mg.L^{-1})	Peak Area of Post Extraction Spike (mg.L^{-1})	Peak Area of Pre-Extraction Spike (mg.L^{-1})	Retention Time (min)	Recovery Percentage (%)
CB 28	0.0104	0.05	0.0604	0.0492	8.071	81.34
CB 52	0.0096	0.05	0.0596	0.0533	9.31	89.42
CB 101	0.0291	0.05	0.0791	0.0811	13.451	102.52
CB 153	0.0146	0.05	0.0646	0.0587	17.309	86.22
CB 138	0.0186	0.05	0.0686	0.0602	18.336	90.67
CB 180	0.007	0.05	0 .0570	0.0511	21.387	89.47
CB 209	0.0039	0.05	0.0539	0.0451	27.727	95.16

Table 8. Physical-chemicals qualities of samples wells.

Sample Wells	Hydrogen Potential pH	Temperature (°C)	Electric Conductivity (ms.cm^{-1})	Piezometric Leve (m)	Material Suspenssion (g.L^{-1})	Concentration of PCBs (ppm)	Location	Specifications Wells
P0	6.4	25	2.8	drill to 1.4	0.009	0.0147	Bou Argoub	Upstream with the presence of an industrial area
P1	6.3	23	3.2	drill to 1.6	0.012	0.026	Belli	-
P2	6.24	25.2	3.32	drill to 9.4	0.0009	0.0052	Miano	In company el-Katibya
P3	6.32	21.2	3.56	drill to 1 m	0.023	0.0387	Om Hechem	rementée Tablecloth
P4	6.44	23	4	drill to 2.35	0.019	0.0417	Bou Charray	
P5	6.32	21.8	5.3	drill to 1.95	0.027	0.0713	Southeast entrance road Soliman Bou Charray	Near the Delice factory and washfactory
P6	6.36	21.9	6	drill to 1.4 m	0.095	0.169	Dowar Louz-Chrifét	Turbid and saline water
P7	6.7	21.5	6.5	drill to 2 m	0.0025	0.0952	Bou-Aamayed	-
P8	6.4	21	7.1	drill to 1 m	0.003	0.014	Sabkha Soliman	saline water

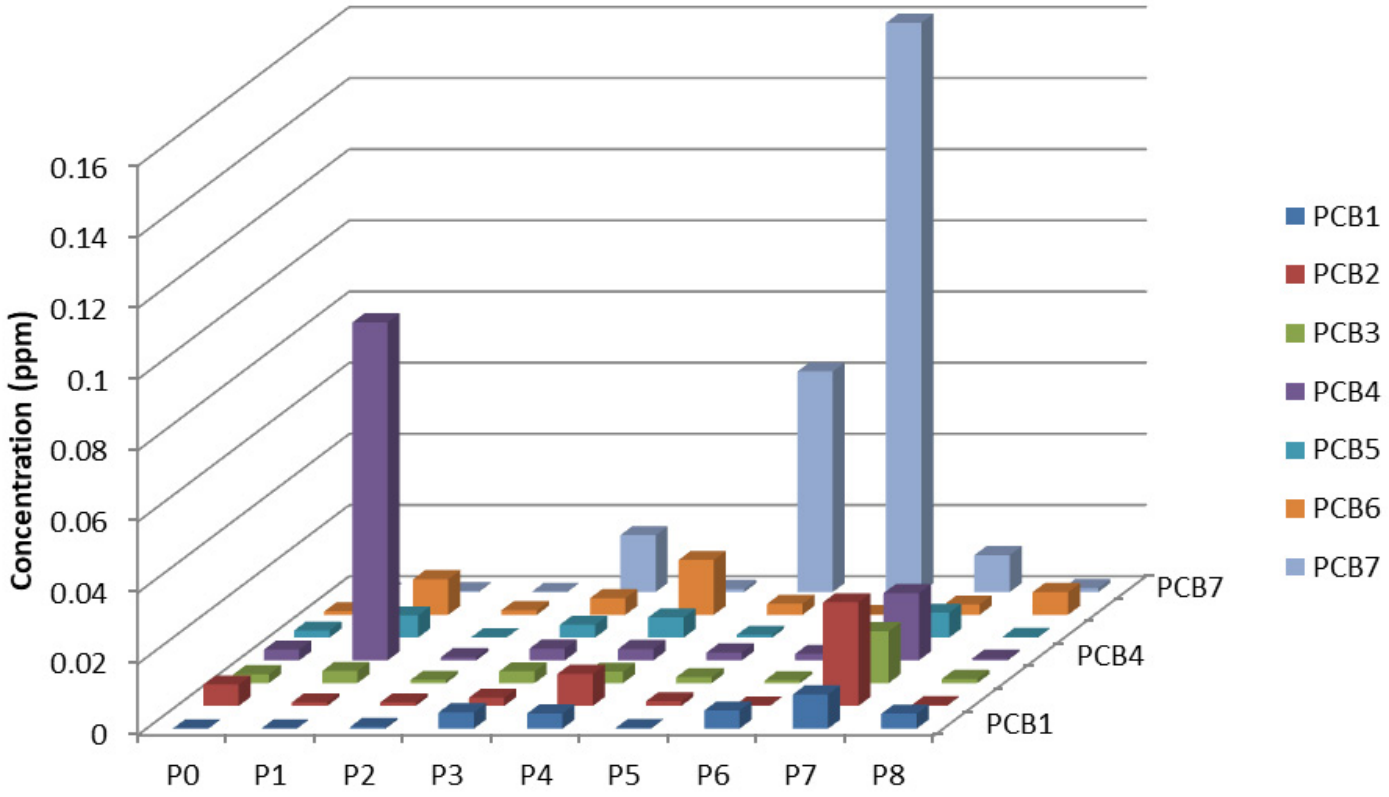

Figure 5. Individual PCBs concentrations in different wells.

Conclusion

To our knowledge, this is the first study to report the residue levels of PCBs in groundwater of Grambalia in Tunisia and provide baseline data for future research, as well, we optimized and validated a novel method of PCBs analysis by gas chromatography coupled with Electron Capture Detector to determine PCBs concentration of water samples wells. Based upon the concentration levels of measured PCBs congeners in water samples, PCBs are currently posing an unacceptable level of risk to ecosystem, agriculture and posing a potential risk to human populations relying on the surface water.

Sources of PCBs must to be identified and managed, industrial wastewater disposal should be strictly monitored and minimize the use of pesticides and organo-chlorinated in agriculture.

Acknowledgment

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