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Search of Organochlorine Pesticide Residues (Pocs) in Bodies of Water in Cotton-Growing Area of Benin by GC-ECD

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ABSTRACT

Despite their incontestable services in agriculture, the use of pesticides is not without consequences on the environment. So, in an attempt to access the impacts of pesticides usage in agriculture in Benin, research of some residual organochloride pesticides have been conducted both in rainy and dry seasons in two cotton growing areas: the northern and central part of Benin. The analytical technique used is GC-ECD. During the dry season we notice that the DDT and its metabolites (DDE and DDD) represent 36% of all organochlorine pesticides (POCs) against 64 % of cyclodiens. Endosulfan comes first representing 57% of all organochlorine pesticides (POCs), then follows DDT with 17 %. During the rainy season these two types of organochlorine pesticides (POCs) represent 73% for cyclodiens and 23% for DDT and its by-products. Endosulfan comes first by representing 47% of all organochlorine pesticides (POCs) followed by DDT representing 12%. In the sediments and regardless of the season, the cyclodiens come first by representing 70% of all organochlorine pesticides (POCs) and then follows the DDT and its by-products which represent 30%. Since in the water column, the sediments are more contaminated in rainy season than in dry season (25273 ppb of all organochlorine pesticide (POCs) in rainy season against 2.256 ppb in dry season), it is derived from this study that northern areas are more contaminated than the central areas. Also a strong correlation has been established between the content of different moleculars of organochlorine pesticide (POCs). This means that the famers still use prohibited pesticides in the two areas despite existing laws like "Stockholm convention" which strictly ban the usage of those moleculars.

Keywords: Organochlorine, residual pesticides, water, cotton area, Benin

1. INTRODUCTION

According to Oerke and Dehne (1997), 42% of the world potential agricultural production is lost because of the plants enemies when pesticides are not used. For example, the European corn borer (Ostrinia nubilalis), an insect of the kind of Lepidoptera, with a family of Pyralidae, now placed in the cramidés, provokes each year a yield loss of one billion dollars American farmers. Weeds cause to Australia annual losses estimated to 3,000 million USD (Silvy, 1995). In Benin, the cotton season 2006-2007 has experienced a downward trend of 50% over the previous year due to the unavailability of endosulfan. This was repeated during the past 2009-2010 cotton campaign. This decreasing data is always accompanied by economic consequences (Colin, 2000).

Despite these great services they have rendered to agriculture, pesticide usage is not safe for the environment. In agriculture, most treatments are applied to the aerial parts of plants (BALDI et al., 1999). However a good proportion of the crop protection product goes directly to the ground and air where they are washed out by precipitation. Water bodies are the main focus of pollutants into the environment. In water, they cause direct and indirect adverse effects on aquatic fauna and even on lands that cannot live without water. According to the World Bank's report (2004), the export incomes of African countries in southern Sahara are dependent on cotton for about 70%. However, cotton cultivation is high synthetic pesticides consuming (Guidou, 1998). Thus, the contamination of natural systems is of great preoccupation in areas of cotton production in southern Sahara African countries including Benin where agriculture involves more than 80% of the workforce (INSAE, 2002).
In Benin, 70% of agricultural inputs are used in central and northern where the majority of farmers are cotton producers (World Bank Report, 2004). In these areas the persistence of pests created a sort of sociological resistance among the population that leads them no to believe the effectiveness of treatment advised by agricultural technicians. So being tired of resistant parasites, but driven by the need to get rid of pests, farmers use banned substances, dangerous and highly persistent which they purchase from the informal markets existing in neighboring countries. The careless observed at borders with neighboring countries and the non-implementation of laws in this field also explain this behavior in Benin (SOCLO, 2008). But the scarcity of adequate Water Supply Facilities (PEA) nearby obliges farmers to use without treatment surface water already contaminated by pesticides, especially in dry seasons.

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Considering the harmful effects of pesticides on human health, on the environment and regarding the need of sustainable development, it is important to develop an approach of ecosystem corrective actions that is to say a comprehensive approach that combines environmental considerations and economic development. This must be based on a diagnosis of the current situation which requires the evaluation of contamination levels of the different environmental compartments. That is what motivates this study.

2. MATERIAL AND METHOD

2.1 - Study Area

2.1.1 - Location

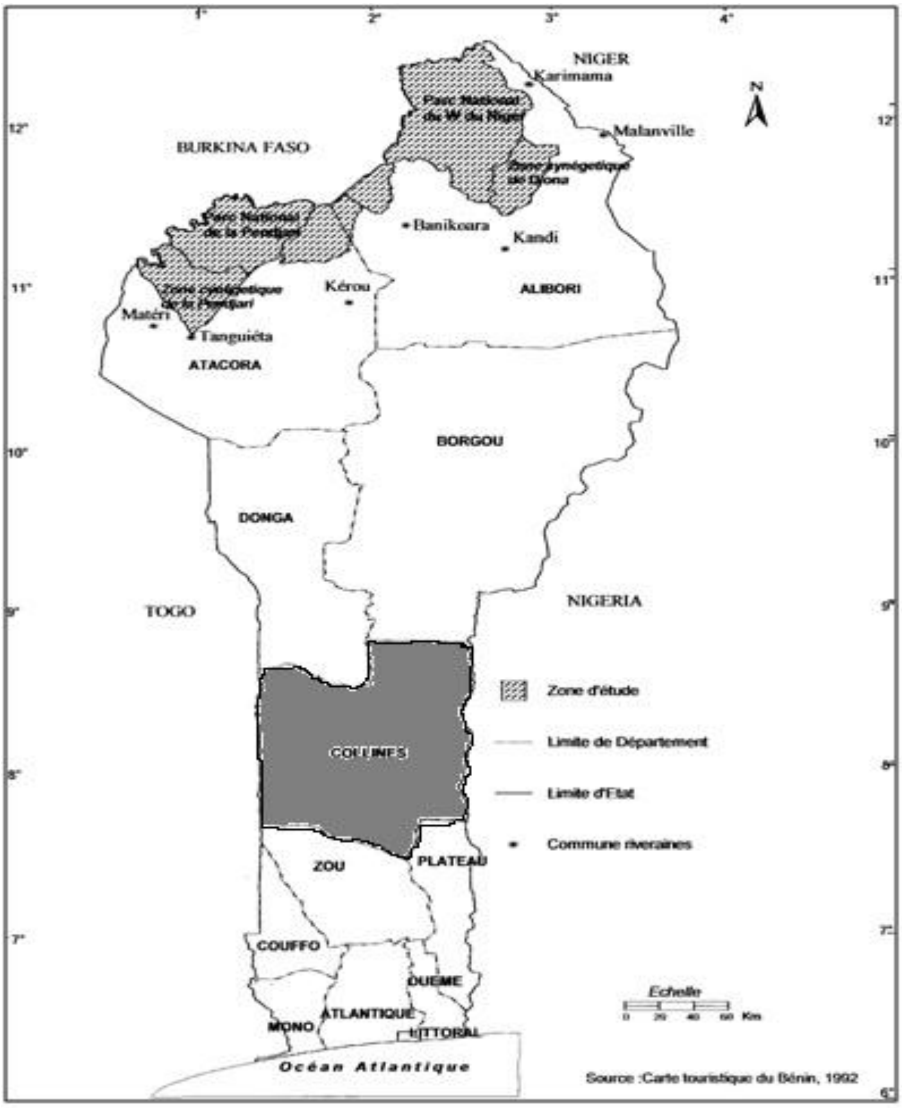

Fig-1: Location of the study areas

The two study areas are located respectively in the central and north-eastern parts of Benin and are colored in grey on the map (Figure 1). In the central part, this study focused on the district of Savalou in the department of Collines. This is between 1° 7' 46" and 3° 34' 56"east longitude and 6 ° 18' 40" and 12 ° 23' 29" north latitude.

In the northern part, the study focused on the Complex Biosphere Reserve W of Niger and Pendjari. This is between 10 ° 30 ' and 12 ° 30' north latitude and between 0 ° 50 ' and 3 ° 17' east longitude.

2.2 *The drainage*

The study area located in central Benin is watered by several rivers. The main ones are Agbado river (at the east) which is the subject of this study. In the west of the central Benin, there are Azokan river and Zou river.

The drainage pattern in the northern consists of the stream Pendjari and its tributaries (Magou, Yatama, Tandjali, Podiéga, Bonkada, Pourou, Pako), river Niger and its tributaries (Mekrou Alibori, Sota) and associated ponds. These ponds are for elephants, hippos, lions, crocodiles… and serve as major sources of water for wildlife, domestic and / or wild in the Complex Biosphere Reserve W of Niger and Pendjari. In dry seasons very few of these are perennial sources of water (Agbossou, 2001).

2.3 *Geological*
In general, these areas are part of the overall crystalline basement of Benin. We observe a low fertility potential due to the phenomena of leaching and soil erosion. Characteristics of the relief requested strong actions to reach the water table (Djaglo, 2003).

Specifically, there are several types of soil namely:
- tropical ferruginous soils impoverished, heavily armored calcite with a surface;
- tropical ferruginous soils with low calcite;
- tropical ferruginous soils, sandy clay or silty clay. These soils are black, thick and are very fertile. They are found around ponds, streams and depressions (Issa, 2004).

2.4 *Cultural activities*
It occupies almost the entire population. The crops are: cotton, maize, fonio, millet, sorghum, yams, rice and other vegetable crops like okra, peppers, etc. The agricultural production techniques used are culture of mounds, ridge, flat, the practice of crop rotation and rotation with fallow. Anxious to increase yields and decrease the need for post-harvest losses, farmers massively and predominantly use synthetic pesticides. This risky behavior generates health and environmental nuisances, respectively for rural and natural ecosystems (Lafia, 1996).

2.5 *Fishing*
Fishing is still traditional in the areas of interest. It uses basic techniques such as hooks, small nets, traps and sometimes toxic plant extracts or synthetic pesticides such as organo chlorine pesticides (POCs) without taking into account that there are other users of the rivers (Elizabeth, 2005). Fishing is practiced by natives and foreigners from Burkina Faso, Togo and Mali.

3. METHOD OF STUDY
3.1 *Identification of sites and sampling campaigns*
Tables 1a and 1b show the sites explored in this study. The criterion for sampling sites selection is based on the fact that the site is either a point of water supply for people or livestock or it is a point of old or recent use of pesticides for the purpose of fisheries, agricultural and / or gardening. In all, twenty fife sites were selected and the two tables bellow display their name and coordinates.

Table-1: Names and Geographical coordinates of sampling sites

N°	Name of the site	Geographical coordinates
01	Mèdétèkpo	N 08°4,584’ E 02°0,1915
02	Dam of SONEB	N07°57,813’ E 002°0,552’
03	Water treatment station of SONEB	N07°55,471 E 002°0,0137’
04	Under the big bridge of Gobada	N07°45,77’ E 001°59,739’
05	A1 (in the river Alibori 200m to the embouchure on the river Niger)	N11º56.015’ E3º17.451’
06	A2 (on the river Niger 200m before the embouchure of the river Alibori)	N11º56.015’ E3º17.451’
07	A3 (On the river Niger 200m after the embouchure of the river Alibori)	N11º56.041’ E3º17.569’
08	PK (Under the bridge Karigui on the river Alibori)	N12°17.335’ E002°56.142’
09	M1 (In the river Mékrou 200m away from the mouth on Niger river)	N12°24.418´ E002° 49. 563´
10	M2 (On Niger river at 200m upstream the mouth of Mékrou stream)	N12° 4.446´ E002°49.548´
11	M3 (On Niger river 200m after the mouth of Mékrou stream)	N12° 24. 44´ E002° 49. 628´
12	Alfa-Koara (W4M)	N 11°26.922’ E 003°04.068’

3.2 *Water samples Collection*

Water samples were collected in amber bottles previously cleaned, dried in an oven at 105 ° C and then tested negative with respect to pesticide residues sought by sampling and analysis of methanol used to condition the sample bottles below described.

On the field, water samples are collected at 25cm below the water surface using weighted bottles. The water thus collected is transferred into another amber bottle (2L) containing 25 mL of methanol. This limits the adsorption of pesticides to the walls of the bottle which is previously labeled.

Table-1b: Names and Geographical coordinates of sampling sites

N°	Name of the site	Geographical coordinates
13	Hunting camp of Djona (W5R)	N 11°39.807’ E 002°54.946’
14	River Kérémou (W6R)	N 11°20.998’ E 002°19.323’
15	Hunting camp of Mékrou (W7R)	N 11°23.993’ E 002°11.703’
16	Mare of Goumori (W8M)	N 11°10.814’ E 002°17.707’
17	Source of elephants (PEN1R)	N 10°53.459’ E 001°30.217’
18	River Tantagou (PEN2R)	N 10°58.901’ E 001°34.176’
19	Mare Bori (PEN3M)	N 10°54.556’ E 001°30.016’
20	River Tanougou (PEN4R)	N 10°48.503’ E 001°26.045’
21	Bridge Magou (PEN5R)	N 11°01.774’ E 001°07
22	Porga bridge (PEN6R)	N 10°59.664’ E 000°58.588’
23	Mare of Tiélé (PEN7M)	N 10°43.609’ E 001°12.483’
24	Bourinissou (PEN8R)	N 10°39.980’ E 001°16.502’
25	Mare of Tiabiga (PEN9M)	N 11°41 E 001°71

3.3 *Collection of sediment samples*

Samples of surface sediment (0-5cm) were collected with a grab Schipeck. They are packaged in aluminum foil previously cleaned with pentane and 95% ethanol using a washed bottle.

3.4 *Storage of samples at the site and laboratory*

Water samples and sediment collected are packed in aluminum foil to protect them against photodegradation. They are placed in coolers where they are kept cool at 4 ° C using ice packs. In the laboratory, water samples are transferred to the refrigerator at 4 ° C while the sediments are frozen in a freezer at -10 ° C until the lyophilization step.

3.5 *Pretreatment of samples*

3.5.1 *Lyophilization*

The sediment samples are lyophilized before analysis. A device freeze CIRP RP2V (Serail, Paris) was used for this purpose. Dry sediments were crushed in porcelain mortar and were sieved at diameter of 2 mm to remove plant debris and pieces of shell in an attempt to get a homogeneous sample. The samples were then transferred to amber bottles tightly closed to protect them from moisture and photodegradation.

3.5.2 *The extractions*

The liquid-liquid extraction was used for all water samples. As for solid matrices, they were extracted by Soxhlet.

3.5.2.1 ***Protocol of liquid / liquid extraction with an organic solvent***
300 mL of water sample were extracted three times successively with 10 mL of pentane using a separating funnel of 1L. After each extraction the liquid is allowed to settle and the organic phase is then extracted in a clean ball. Once the three organic extracts obtained, the ball is placed in a rotary evaporator Heidolph type for the concentration at 1 ml.

3.5.2.2 ***Extraction by Soxhlet***
This is a solid-liquid extraction which enriches slowly the solvent of the ball in molecules to be analyzed while refueling the sample to be extracted with freshly distilled solvent. The Soxhlet extractor containing one paper cartridge “wattman” filled with 30 mg freeze-dried sediment is mounted on a ball neck of 250 mL filled at 2/3 of pentane. By heating, the solvent is evaporated to a refrigerant through the extractor. Once condensed, the solvent falls back in the extractor where it accumulates in contact with the sample to be extracted. After a while, the level of solvent in the extractor reaches the top of "U" inverted siphon. It is then withdrawn from the extractor to the ball and is recycled. After 8 hours of extraction, the content of the ball taken and is then concentrated in a same rotary evaporator at 1 ml.

3.6 ***Step of Purification***
The different treatments previously performed during the extraction can eliminate a significant number of organic compounds capable to interfere with pesticides, but the capillary column used in gas chromatography, whatever its efficiencies, does not separate all the targeted compounds, thus does not allow to obtain pure individual chromatographic peaks. For this, the open column chromatography on florisil coupled with a step of the extract desulphurization was carried out by introducing at the end of the column an activated copper layer to refine the preparatory steps of pesticides analysis. In fact, the sedimentary and/or biological matrixes often contain a significant amount of elementary sulfur which interferes in the chromatographic analysis, particularly in the case of the one coupled with EDC (Tapie, 2006). The florisil column chromatography helps to separate organochlorine compounds in three fractions (Soclo, 2008).

Fraction I, obtained after elution with 17.5 ml of hexane contain HCH, heptachlor, the op-DDE, the pp'DDE, and the PAHs;

Fraction II, resulting from the elution with 12.5 ml of hexane and dichloromethane (3: 2 by volume) may contain molecules pesticide such as alpha-HCH, lindane, the opDDD the pp'DDD the op'DDT the pp'DDT and the toxaphene;

Finally, the fraction III obtained after elution with 10 ml of dichloromethane, d contain alpha-endosulfan, dieldrin, endrin, beta-endosulfan and endosulfan sulfate.

The fractions were purified and re-concentrated using nitrogen and taken back in isooctane. 200µl of each extract was taken in a pillulier and conserved for analysis with the use of gas chromatography.

3.7 ***Analytical equipment***
Analyses were performed using a gas chromatograph Hewlett Packard type 5890 Series II equipped with:

- two injectors: an on-column injector and a split / splitless injector;
- two detectors, one for flame ionization (FID) and the other for electron capture (EDC). This last detector is used for the determination of organochlorine pesticides because of its selectivity for chlorinated compounds. It is very sensitive, but has a range of linearity response which is not very high, that is 10^2 to 10^{-9} (Tranchant, 1995). The method of peak identification is to compare the relative retention times with those standards.

The chromatogrph downstream is coupled to an integrator-type calculator Hewlett Packard 3396A to integrate chromatographic peaks and to determine the concentrations of individual pesticides sought.
For this study the on-column injector was selected and EAD as a detector. The choice of this injector is based on the fact that it does not leak and that all the amount of sample injected passes onto the column. The one chosen is a silica capillary column, type SUPELCO, INC, which is 30 meters long and 0.53 mm in diameter and which grafted phase is a thin film of 608 SBP of 0.5 µm. This type of column is recommended because of its chemical inertia vis-à-vis of compounds containing chlorine. It is also recommended because of its stability and its tolerance vis-à-vis of temperatures above 300 °C. Each time, 1µl of sample analyzed was injected.
The vector gas used was the nitrous with analytical quality and purity of 9.999999 or 9.6.
The conditioning of the column is an essential preliminary step to any analysis: it eliminates the possible contamination of the column. To that end, the detector is heated up to 300 ° C, the oven and the injector up to 200 ° C for 48 hours without interruption. The column is ready for analysis when the detector signal is less than 50 (value read on the display screen of the chromatograph). The displayed value of the signal allows doing the following classification:

15 <signal (ECD) <50: desirable.
50 <signal (ECD) <100: acceptable.
signal (ECD)> 100: inadmissible.

In the framework of this work, the signal value is often between 18 and19 which indicates a good conditioning of the column and a negligible level of contamination.

To avoid destruction of the column, it is advisable to let flow permanently the carrier gas when one of the compartments of the chromatograph is being heated.

For analysis, two internal standards were used for all the organochlorine pesticides studied: the PCB 198 for heptachlor, aldrin, 2.4 '-DDE and 4.4'-DDE and DDT perdeuterated (4.4 '-DDT-d8) for the other research pesticides.

4. QUALITY ASSURANCE QUALITY CONTROL (QA / QC)

4.1 *Validation of analytical methods*

To enable judicious use of data collected as a result of chemical analysis, we passé through validation, as it has been done in several similar studies on organic micro pollutants in natural systems (Thompson, 1999; Tapie, 2006; Soclo, 2008).

4.2 *Validation of quantification solutions for the internal calibration and analysis*

It consisted of regular analysis by GC-ECD of two reference solutions, containing the desired compounds and internal standard at concentrations of about 0.25 $\mu g.g^{-1}$ isooctane. This served to validate the reference solutions and then led to the calculation of response factors of individual compounds in relation to their internal standard.

If the first solution used as a reference solution allows the calculation of response factors of individual compounds in relation to their internal standard, the second solution is used as unknown pseudo sample to validate the solutions of quantification.

Concerning the analysis validation, before, in the middle and after each analytical sequence, reference solutions containing the internal standards are injected. Three replicas per sample were collected for both the contamination controls and the samples. The averages are then calculated as well as standard deviations, which were used to estimate the precision of the results. Thus, the solutions of quantification and analysis are validated for differences not exceeding 20%.

4.3 *Witnesses of contamination*

In order to ensure the quality of analysis, contamination controls were designed to verify the absence of contamination due to protocol preparation, operator, equipment used and the work environment. For each set of samples, a contamination control is analyzed in parallel with samples. In a practical manner, the analytical protocol is implemented without any sample, that is to say, without any solvents or products that may be in contact with the sample during the whole analysis: all the way from the stage of internal standard doping up to the stage of quantification with an intermediate stages of extraction, concentration, purification and fractionation according to the types of analysis considered.

Thanks to contamination control, the values recorded from each analyzed sample were corrected by subtracting the average value of contamination levels from value obtained from each sample.

4.4 *Double calibration and operating performance*

Used as a standard of performance, octachloronaphtalene (OCN) was added to each sample to quantify the internal standards initially introduced in the protocol. This helps to evaluate the percentage of recuperation. It is a actually a double calibration in the quantification of pesticides which aims to strengthen the quality of analysis. Our analysis were considered valid in the case the recuperation rates are higher than or equal to 80%, otherwise they analysis is repeted.

4.5 *Limits of methodological detection for the matrices analyzed and linearity range of the detector EDC used*

The limits of methodological detection correspond to the lowest measurable values by the analytical method used. They are measured from the compound response in the background noise to which where they represent the triple. EDC is a detector that the response is proportional to the concentration of electrophilic compounds that go through it. It is very sensitive; with detection limits for polyhalogenated compounds, generally ranging in the picogram (10-12 g) domain, or fentogramme (10-15 g) domain. Lindane ($_{\gamma}$-HCH) organochlorine pesticide with six (06) atoms of chlorine is commonly used to test the sensitivity of ECD (Pierard, 1995).

The linearity range of the liquid matrix was determined using a standard reference solution consisting of an equimolar mixture of p, p'-DDT, p, p'-DDE $_{\gamma}$-HCH, α-Endosulfan and heptachlor. This blend is made from individual reference solutions with a concentration of 500 ng / uL obtained from Professor Villeneuve at the University of Bordeaux I. Dilutions ranging from 10^2 to 5×10^5 of the initial mixture were prepared and injected. Curves giving the area of each pesticide according to the injected mass are plotted in order to detect the area of linearity of the detector response. Only curves with correlation coefficients R^2 exeeding 0.9960 with at least 06 points are accepted. This is particularly important because, despite the proportionality of the EDC with the molecular concentration, the linearity range is slightly extended (2 to 3 orders of magnitude).

In the absence of a certified sediment sample, 500 g of coastal marine sediment from the beach of Fidjrossè heated up to 625 ° C for 24 h, desalinated by residing 24 hours three times in MilliQ water, was used as a reference matrix sediment. 30 g of sediment was contaminated with 60µL of the mixture of reference undiluted pesticides of 500 ng / uL. The extract of this sediment was used to determine the detection and quantification limits of solid matrices.

5. STATISTICAL ANALYSIS OF DATA

To highlight the possible relationships between the various parameters measured, we draw correlation matrices using Excel. The calculation of the sum of the contents of pesticide molecules and the sum of relative proportions helped to establish the possible relationship existing between the levels of water contamination and sediments studied. This helps to determine the major chemical indicators of pollution by residual pesticides in the study areas.

6. Results and discussion:

6.1. *Calibration data of the analytical equipment*

Table-2: Calibration Parameters

Pesticide	R^2	Zone of linearity ($pg.\mu L^{-1}$)	Limits of detection LOD ($ng\ mL^{-1}$)		Limits of quantification LOQ ($ng\ mL^{-1}$)	
			Liquid matrix	**Matrix solid**	**Liquid atrix**	**Matrix solid**
P,p'-DDT	0.9978	150-350	1.25	1.58	4.30	4.30
γ-HCH	0.9962	150-350	1. 28	2.41	3.50	6.56
Heptachlor	0.9982	150-350	1.02	2.32	2.80	6.31
α-Endosulfan	0.9998	150-350	0.10	0.5	0.28	1.32
p,p'-DDE	0.9992	150-350	1.32	1.67	3. 60	4.65

From the analysis of the data in Table 2 we can see that the correlation coefficients R^2 of calibration curves used vary between 0.9962 and 0.9992, with a linear domain that ranges between 150 and 350 pg / µl. Overall our methodological detection limits are between 0.1 and 1.3 ppb for liquid matrix and 0.5 to 2.4 ppb for solid matrices. The limits of quantification vary between 0.30 and 4.30 ppb for liquid matrices and between 1.30 and 6.50 for solid matrices. These results served as guide parameters for field investigation.

6.2. *Results of natural samples analysis*

Table-3: Content in individual pesticide molecule detected in water samples per study site

	Dry season (Average± σ)								Rainy season (Average±σ)							
	p,p'-DDT	**p,p'-DDE**	**p,p'-DDD**	**Endosulfan.**	**Lindane**	**Dieldrine**	**Heptachlore**	**POCs totaux**	**p,p'-DDT**	**p,p'-DDE**	**p,p'-DDD**	**Endosulfan.**	**Lindane**	**Dieldrine**	**Heptachlore**	**POCs totaux**
Mèdétèkpo	8	23	0	0	0	0	0	31	21	49	14	35	0	0	0	119
Dam SONEB	17	45	0	0	0	0	0	62	18	37	9	28	0	0	0	92
Station SONEB	0	0	0	0	0	0	0	0	5	13	3	8	0	0	0	29
Grand Bridge Gobadi	0	0	0	14	0	0	0	14	32	26	17	19	0	0	0	94
Bridge Karigui	12	9	14	0	0	0	0	35	16	32	25	0	0	0	0	73
A1	0	0	0	0	0	0	0	0	14	25	10	13	0	0	0	62
A2	0	0	0	0	0	0	0	0	23	54	25	8	0	0	0	110
A3	0	0	0	0	0	0	0	0	0	0	0	16	0	0	0	16
M1	15	70	18	24	0	0	0	127	35	72	16	176.78	189.23	188.56	0	677.57
M2	18	65	23	12	0	0	0	118	22	63	21	137.84	140.23	139.45	0	523.52
M3	15	73	35	8	0	0	0	131	30	80	23	368.66	370.32	369.26		1241.24
PEN1R	0	0	0	0	0	0	0	0	0	0	0	168	0	0	0	168

PEN2R	0	0	0	0	0	0	0	0	34	0	0	203	0	0	0	237
PEN3M	0	0	0	0	0	0	0	0	40	6	0	300	38	0	0	384
PEN4R	0	0	0	0	0	0	0	0	0	0	0	414	0	0	0	414
PEN5R	44	13	9	430	7	0	10	513	86	7	4	46	15	0	29	187
PEN6R	79	13	0	280	19	27	5	423	128	0	0	115	42	15	37	337
PEN7M	14	0	0	128	0	0	0	142	0	0	0	0	0	0	0	0
PEN8R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PEN9M	0	0	0	0	0	0	0	0	86	0	0	0	32	0	43	161
W4M	0	0	0	34	0	0	0	34	0	0	0	217.6	0	0	0	217.6
W5R	18	0	0	162	0	0	0	180	78	0	0	35	0	0	23	136
W6R	72	18.4	0	136	29.8	2.8	0	259	63.6	18	0	286	0	0	7.6	375.2
W7R	71	4	0	45	0	0	44	164	0	0	0	156	0	0	0	156
W8M	0	0	0	23	0	0	0	23	0	0	0	52	0	0	0	52
POC totaux	383	333.4	99	1296	55.8	29.8	59	2256	731.6	482	167	2802.88	826.78	712.27	139.6	5862.13

Table-4: Content in individual pesticide molecule detected in sediment samples per study site

	Dry season (Average ± σ)								**Rainy season (Average± σ)**							
	p,p’-DDT	**p,p’-DDE**	**p,p’-DDD**	**Endosulfan**	**Lindane**	**Dieldrine**	**Heptachlore**	**POCs totaux**	**p,p’-DDT**	**p,p’-DDE**	**p,p’-DDD**	**Endosulfan**	**Lindane**	**Dieldrine**	**Heptachlore**	**POCs totaux**
Mèdétèkpo	178	300	82	20	3	4	2	589	89	110	28	54	9	4	0	294
Dam SONEB	56	80	25	45	5	3	5	219	364	278	37	47	12	9	0	747
Station SONEB	20	16	12	15	3	2	0	68	25	56	10	25	2	3	0	121
Grand Bridge Gobada	23	32	8	48	0	0	0	111	96	88	9	56	4	6	0	259
Bridge Karigui	40	95	19	15	6	2	5	182	160	154	12	280	29	58	12	705
A1	50	62	20	40	9	4	3	188	128	176	15	325	56	19	20	739
A2	45	70	25	26	12	3	2	183	156	125	23	289	46	33	16	688
A3	86	30	23	0	0	0	6	145	148	152	24	405	63	36	13	841
M1	80	48	16	25	7	0	5	181	259	129	28	180	24	28	18	666
M2	90	35	31	27	10	0	7	200	372	170	36	140	32	26	10	786
M3	85	32	34	36	4	0		191	359	165	26	203	17	30	14	814
PEN1R	0	0	0	69	0	0	0	69	0	0	0	48	0	0	0	48
PEN2R	0	0	0	248	0	0	0	248	0	0	0	203	0	0	0	203
PEN3M	0	0	0	523	0	0	0	523	0	0	0	376	0	0	0	376
PEN4R	0	0	0	164	0	0	0	164	0	0	0	157	0	0	0	157
PEN5R	447	34	26	1463	100	68	96.3	2234.3	532	37	29	1235	135	87	122	2177
PEN6R	1013	239	67	5748	234	89	263.7	7653.7	1244	276	78	6054	250	97	288	8287
PEN7M	129	11	0	715	0	0	0	855	146	6	0	936	0	0	0	1088
PEN8R	0	0	0	88	0	0	0	88	0	0	0	102	0	0	0	102
PEN9M	0	0	0	0	0	0	0	0	0	0	0	88	0	0	0	88
W4M	0	0	0	325	0	0	0	325	0	0	0	301	0	0	0	301
W5R	99	31	5	1620	113	0	98	1966	117	40	9	1845	146	0	138	2295
W6R	183	18	10	1160	88	0	65	1524	123	0	0	687	64	0	34	908
W7R	536	28	51	897	47	0	12	1571	604	35	44	956	65	0	39	1743

W8M	90	36	0	528	0	0	0	654	115	38	0	687	0	0	0	840
POC totaux	3250	1197	454	13845	641	175	570	20132	5037	2035	408	15679	954	436	724	25273

Table-5: Content in total POC and relative proportion of each pesticides molecular researched in the matrices

Iindividual molecular pesticides	POC Water from Dry season	% POC	POC water from Rainy season	% POC	POC Sediments from Dry season	% POC	POC Sediments from Rainy season	% POC
p,p'-DDT	383	17	731.6	12	3250	16	5037	20
p,p'-DDE	333.4	15	482	8	1197	6	2035	8
p,p'-DDD	99	4	167	3	454	2	408	2
Endosulf.	1296	57	2773.88	47	13845	69	15679	62
Lindane	55.8	2	826.78	14	641	3	954	4
Dieldrine	29.8	1	712.27	12	175	0.8	436	2
Heptachlore	59	3	139.6	2	570	3	724	3
Total	2256	100	5833.13	100	20132	100	25273	100

6.2.1 *Data analysis*

The analysis of data in Table 5 helped doing some of observations. First, in the dry season it is noted that DDT and its metabolites DDE and DDD represent 36% of total POCs of water, against 64% for cyclodiene. The Endosulfan comes first by representing 57% of total POCs, and then follows DDT which represents 17%. In rainy season, these two families of pesticides represent 73% for cyclodienes and 23% for DDT and its derivatives. Endosulfan, the first of cyclodienes, represents 47% and is followed by DDT that represents 12%.

Also, we notice that the water column is more contaminated in the rainy season than it is in the dry season (the total POCs content in the dry season is 2256 ppb in the water column against 5833 ppb in the rainy season).

In the sediments, cyclodienes come first by representing more than 70% of total POCs against 30% for DDT and its derivatives regardless of the season. As in the water column, sediments are more contaminated in the rainy season than in the dry season: 25 273 ppb in total POCs against 2256 ppb.

Both in the dry and rainy seasons the sediments are more contaminated than the water column in total POCs (20,132 ppb in sediments against 2256 ppb in the water column during the dry season, in contrast, during the rainy season the content in total POC is 25,273 ppb in the sediments against 5833 ppb in water).

From the Analyzing of data in Table 6 for related to the levels of contamination of the different sites studied, it appears that the contents of POCs in the water column during the dry season range from background noise (not determined - nd) to 513 ppb at 'pont de Magou' (PEN 5R) that is 23% of total POCs. In rainy season, these contents vary also from the background noise to 1241 ppb at 'l'embouchure de la Mékrou' located in Niger river (site M3). This content represents 21% of total POCs found in the water column during this period. None of these contents has reached the guideline values of 2000 ppb for individual molecules recommended for drinking water by the World Health Organization (WHO) conrning the DDT, lindan and dieldrin. On the other hand, these contents by far exceed sometimes the standards of the European Union that require a threshold of 0.1 ppb for individual molecule of pesticides and 0.5 ppb for the sum of residual contents detected.

In sediments, POCs are detected in almost all samples, especially in the rainy season. The distributions of organochlorine pesticides molecules observed in water samples are also observed in the sediments of the same sites. POCs rates in sediments are much high compared to those measured in water samples, this could be explained by the hydrophobia (low aqueous solubility) of organochlorines, which preferentially adsorbed on suspended particles in water to accumulate in sediment where anaerobic conditions favor their preservation against any degradation. By a comparative analysis of sites, we notice that the site 'pont de Porga', built on the river Pendjari, is the most contaminated with 7654 ppb in the dry season. That is 38% of total POCs found in sediments and 8287 ppb in the rainy season corresponding to 33% of the total POCs recorded at sites sampled during this period.

No site in the catchment of the Agbado river (central Benin) presents the proportions of pesticides exceeding 3% of total POCs recorded, regardless of the analyzed matrix and the season. It can therefore be inferred that the sites in northern Benin are more contaminated than the sites in the central Benin.

In northern ecosystems, particularly in the biospheres of Pendjari and W of Niger, the POCs contents ponds are below 4% regardless of the matrix or the season, while water from rivers concentrate most of CSWS detected. One could attribute this predominance of pesticides in rivers, not only to the high extent of the watersheds of these rivers but also to the turbulence of their water that causes charged surface sediments to be returned continuously into solution.

Table-6: Contamination levels by investigated site.

SITES	POC Water-Dry season	% POC - Dry season	POC water-Rainy season	% POC Rainy season	POC Sediments-Dry season	% POC Dry season	POC Sediments-Rainy season	% POC Rainy season
Mèdétèkpo	31	1	119	2	589	3	294	1
Dam SONEB	62	3	92	2	219	1	747	3
Station SONEB	nd	nd	29	0.5	68	0.3	121	0.5
Grand Bridge Gobadi	14	0,6	94	2	111	0,5	259	1
Bridge Karigui	35	1	73	1	182	0.9	705	3
A1	nd	nd	62	1	188	0.9	739	3
A2	nd	nd	110	2	183	0.9	688	3
A3	nd	nd	16	0.3	145	0.7	841	3
M1	127	6	677.57	12	181	0.9	666	3
M2	118	5	523.52	9	200	1	786	3
M3	131	6	1241.24	21	191	0.9	814	3
W4M	34	1	217.6	4	325	2	301	1
W5R	180	8	136	2	1966	10	2295	9
W6R	259	11	375,2	6	1524	7	908	3
W7R	164	7	156	3	1571	8	1743	7
W8M	23	1	23	0.4	654	3	840	3
PEN1R	nd	nd	168	3	69	0.3	48	0.2
PEN2R	nd	nd	237	4	248	1	203	0.8
PEN3M	nd	nd	384	6	523	2	376	1
PEN4R	nd	nd	414	7	164	0.8	157	0.6
PEN5R	513	23	187	3	2234.3	11	2177	9
PEN6R	423	20	337	6	7653.7	38	8287	33
PEN7M	142	6	nd	nd	855	4	1088	4
PEN8R	nd	nd	nd	nd	88	0.4	102	0.4
PEN9M	nd	nd	161	3	nd	nd	88	0.3
POC total	2256	100	5833.13	100	20132	100	25273	100

This can affect the risk of poisoning wildlife and / or household that uses these sources of water for drinking purposes as Soclo et al. (2003) showed through studies conducted on the same ecosystems. To deepen the analysis, correlation matrices were drawn between the contents of different POCs obtained from each matrix to identify possible links between contents and types of residues found (see Tables 7, 8, 9 and 10 below).

Table-7: Correlation matrix of data from water samples in dry season

	p,p'-DDT	p,p'-DDE	p,p'-DDD	Endosulf.	Lindan	Dieldrin	Heptachlor
p,p'-DDT	1	0.12	-0.03	0.70	0.90	0.83	0.57
p,p'-DDE		1	0.81	-0.08	0.00	-0.01	-0.07
p,p'-DDD			1	-0.02	-0.13	-0.13	-0.09
Endosulf.				1	0.67	0.64	0.27
Lindan					1	0.87	0.23
Dieldrin						1	0.23
Heptachlor							1

Table-8: Correlation Matrix in rainy season water

	p,p'-DDT	p,p'-DDE	p,p'-DDD	Endosulf.	Lindan	Dieldrin	Heptachlor
p,p'-DDT	1	-0.04	-0.09	0.00	0.09	0.02	0.88
p,p'-DDE		1	0.89	0.12	0.71	0.73	-0.29
p,p'-DDD			1	-0.08	0.50	0.53	-0.29
Endosulf.				1	0.44	0.43	-0.12
Lindan					1	0.99	0.02
Dieldrin						1	-0.12
Heptachlor							1

Table-9: Correlation matrix of data of sediment dry season

	p,p'-DDT	p,p'-DDE	p,p'-DDD	Endosulf.	Lindan	Dieldrin	Heptachlor
p,p'-DDT	1	0.50	0.59	0.90	0.88	0.75	0.88
p,p'-DDE		1	0.86	0.44	0.42	0.25	0.44
p,p'-DDD			1	0.38	0.41	0.20	0.40
Endosulf.				1	0.95	0.68	0.97
Lindan					1	0.66	0.98
Dieldrin						1	0.75
Heptachlor							1

Table-10: Correlation matrix sediment rainy season

	p,p'-DDT	p,p'-DDE	p,p'-DDD	Endosulf.	Lindan	Dieldrin	Heptachlor
p,p'-DDT	1	0.58	0.87	0.85	0.83	0.68	0.84
p,p'-DDE		1	0.78	0.34	0.40	0.30	0.34
p,p'-DDD			1	0.61	0.67	0.40	0.62
Endosulf.				1	0.90	0.63	0.96
Lindan					1	0.66	0.97
Dieldrin						1	0.69
Heptachlor							1

The analysis of the correlation matrix of the content of residues of individual pesticides molecules of water samples in the dry season or rainy season (Tables 7 and 8) reveals a strong correlation between p, p'-DDT, endosulfan, lindane and dieldrin. There is also a strong correlation between p, p'-DDE and p, p'-DDD. In addition, endosulfan is highly correlated with Lindane and Dieldrin.

The same correlations noted in water samples are found in sediment samples (Tables 9 and 10), showing that the POCs molecules detected are all correlated.

6.2.2 *Discussion*

Among the POCs detected, two molecules are dominant: Endosulfan and DDT. These observations are in line with those made in 2003 by Soclo et al. and show that these two molecules with heptaclore predominate in aquatic ecosystems of the Complex Biosphere Reserve W of Niger and Pendjari in the northern Benin. The presence of DDT and its metabolites in the water column during the dry season led to calculate the ratio of DDT / (DDD + DDE + DDT) in order to judge whether the parent compound has been used recently or not. The observed values range from 0 and 0.5 regardless of the matrix and the season considered. This ratio indicates a predominance of DDT on each of these degradation products, and its presence in the water column during the dry season reflects its recent contribution in the water. This then confirms the hypothesis that POCs prohibited continue to be imported fraudulently and used in Benin in agriculture, gardening or for other purposes in the vicinity of water bodies. These present analyzes come out with the same trends noted by Elisabeth et al. (2006a and 2006b) at the Ouémé river and its tributaries in Benin. Ioannis et al. (2006) on rivers and lakes of Greece has also noticed the presence of organochlorine pesticides in water bodies with values reaching or exceeding tolerable limits in some places.

On the contrary, Godfred et al. (2008) have found in the lake Bosomtwi in Ghana content of about 0.061 ± 0.03 ng g-1 in sediments and 0.012 ± 0.62 ng g-1 in water. These two contradictory aspects of the state of aquatic ecosystems in terms of pesticide pollution in both countries in the south and countries in the north, show that beyond the strict regulations, only keeping track on the ground, allows to really apprehend the true health state of targeted ecosystems and the effectiveness of protection.

The strong correlations between the POCs molecules, noted both in water and in the sediment, regardless of the season, may have to do with the fact that farmers import inputs prohibited by the laws as they are tired of pest pressure and had become resistant to sociological methods recommended by the technicians in agriculture field. When using pesticides, they perform some unorthodox chemical associations comprising endosulfan and other POCs prohibited. The aim of farmers in behaving that way is twofold: the first is to get the desired and immediate effect on pests and second, is to trick the vigilance in case of control by pretending to use simple and non-prohibited endosulfan. The relative abundance of POCs in rivers compared to ponds, showed that runoff and streams tributaries are important corridors for residual pesticides, this abundance is also reinforced by fishing where fishermen adopted the usage of banned pesticides increase their productivity.

7. CONCLUSION

In sum, the results from gas chromatography on water or sediment samples in dry and rainy seasons confirm the predominance of DDT and endosulfan in all the researched POCs. The interdependence of the molecules of p, p'-DDT, p, p'-DDE, p, p'-DDD, endosulfan, lindane, heptachlor and dieldrin would be linked to vegetable treatments by unorthodox usage of POCs pesticides that are prohibited in areas of cotton cultivation and gardening.

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