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## RESEARCH ARTICLE

# ASSESSMENT OF CERTAIN ORGANOCHLORINE PESTICIDE AND POLYCHLORINATED BIPHENYLS IN SEDIMENTS - UPSTREAM PART OF SEBOU RIVER - MOROCCO

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**ABSTRACT**

The main purpose of this work was to investigate for the first time, levels of certain organochlorine pesticides and polychlorinated biphenyls in Sebou river sediments in its upper part. Three sampling campaigns were carried out in three years between October 2018 and October 2020 in six sampling locations of the river. The results of our investigations have showed that the sediments contains more or less pollutants depending on the sampling area. The downstream part of the studied area is more loaded with OCPs and PCBs than the upstream part. HCH and DDT isomers were two dominant pollutants of OCPs in this region. Levels of ∑3HCHs were ranging from 0.56 to 3.87 ±0.15 ppb and ∑3DDTs concentrations ranging from 0.96 to 5.30 ±0.10 ppb. For individual HCHs, α-HCH registered the highest value of 2.31 ppb, and β-HCH was the most dominant isomer in all stations, and p,p'-DDT was the most dominant with 2.61 ppb. The concentrations of ∑5PCBs in sediment samples were between 1.91 and 6.53 ±0.20 ppb. The maximum levels of these pollutants were recorded in the sediments sampled from Allal El Fassi dam. The presence of these molecules could be directly related to the use of pesticide upstream and to aerial deposition due to their global movement.



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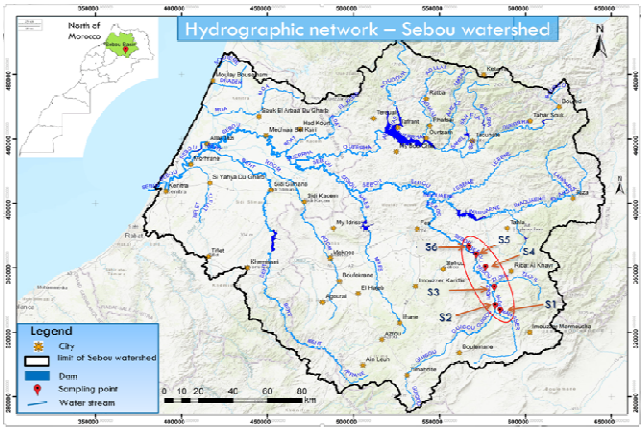
# INTRODUCTION

Organochlorine pesticides (OCPs) and (PCBs) are typical persistent organic pollutants, with important bioaccumulation potential in environmental schemes (1).They are banned by the Stockholm Convention since 2001 and listed as primary pollutant concerns (2). Due to their long range atmospheric transport, toxicity, persistence, bioaccumulation by food chains and adverse effects on environmental and biological wellbeing (3, 4). These compounds have caused global concern as poisonous environmental contaminant(5-7) and are regularly detected in a large variety of matrices such as water, soils, sediments, biota and air, in non-industrial and industrial areas (8, 9).Theyattract scientific and governing interests due to their high production volumes, their persistence, and their potential damaging effects on non-target organisms (4, 10, 11) Persistent organic pollutants can be transported from pollution sources to aquatic ecosystems through various pathways including atmospheric transport and deposition, indirect and direct discharge, and riverine inputs (5, 12-14). They tend to separate strongly from particles and settle in the water column to the sediment because of their hydrophobic properties. They tend to separate strongly from particles and settle in the water column to the sediment because of their hydrophobic properties. Therefore, the sediment is considered as their temporary or final sink (15, 16).

Under other conditions, resuspension process in estuarine and near coastal environments can acts as a source of contaminants in the water column (17).Consequently, sediments in the aquatic environment are considered to be one of the most important incidence of OCPs and PCBs. In consideration of high toxicity and possible hazards of OCPs and PCBs, the exploration of POPs concentrations in sediments is necessary to distinguish historical or anthropogenic sources and reveal potential ecological threats to the aquatic environment (18, 19).Additionally, it is needed to comprehend the spatial-temporal dissemination characteristics, as well as sources analysis of POPs in the riverine sediments for environmental pollution control (5).




**Map 1. Sampling points on the hydrological map of the Sebou watershed**

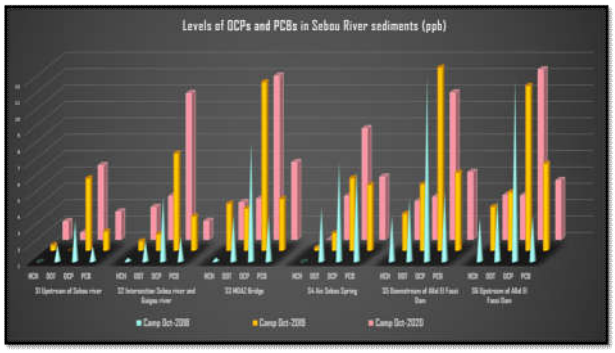



**Figure 1. OCPs and PCBs levels in sediments in Sebou river (ppb)**

The Sebou river, is one of major river in Morocco, draining the third of masse volume water in the country (20),situated close to the one of most urbanized and industrialized areas and receiving raw waste waters from a population of nearly 2.6 million inhabitants, Waters of Sebou river are used for a varied range of purposes from which the most relevant is the irrigation of numeroussorts of cultures, which is subsequently associated to non-negligible threats. Some OCPs were extensively used betweenthe sixteen and the eighteen in Morocco mutually for agricultural and sanitary purposes. In fact, these compounds were banned since 1984 in Morocco except for lindane (γHCH) and DDTs that are tolerated in public health emergencies (21). Many authors have been investigating the occurrence and distribution of POPs in environmental matrices around the world. In Morocco few studies have been conducted (19, 22-24)to assess levels of OCPs and PCBs in marine sediment, but to our knowledge, there are no reported studies in Morocco relating to the pollution of sediments of the upper Sebou river by organochlorine pesticides and polychlorinated biphenyl. This work constitutes to our knowledge the first report about the presence of OCPs and PCBs in sediments from different locations in the upper part of Sebou river. It aims to assess their levels and their temporal variations and interactions with the different physico-chemical parameters, attempting to provide a baseline for further studies of these pollutants in environmental ecosystems.

## MATERIAL AND METHODS

**Site description and sampling:** The study was carried out in northwest Morocco, in the Sebou Basin. It is a big catchment with an overall surface area of 40000 km$^2$, covering near 6% of the Moroccan national territory and 6.25% of the useful agricultural area of the country. Due to its favourable climate and fertile soils, a wide range of crops (olive trees, sugar beet, wheat and sunflower) are cultivated in the area, increasing thus the quantities of pesticides in the environment. Our study area is located upstream of Fez city which is the biggest town in Sebou basin. The city of Fez is responsible for 40% of the total impact of water quality on the Sebou river (20, 25 - 27) and this includes industrial effluents generated by many industries including tanneries, metal works, potteries. Every day significant volumes of chemicals are flushed into the river increasing the contamination of all environment compartments. Our study area represents a background site located 50 Km upstream of Fez city, were industrial activities give way to agriculture. A systematic and grid sampling method was favoured to perform small scale and long term (the upper Sebou river for three consecutive years) monitoring, specially to evaluate temporal tendencies of contamination. The impact of seasonal variability was reduced. The monitoring was conducted in the same period for 3 years. September-November period was selected for several reasons that matches with the end of the growing season, which is appropriate for monitoring sediments because there are not recent uses of pesticides which permits to establish what pesticides are regularly present in the environment because of their ability of accumulation and persistence. Sediments samples were collected at 6 points along the upper Sebou river, the upstream point is located 50km away from the last point. The samples were collected for 3 consecutive years 2018, 2019 and 2020, they were composed by scooping with a stainless-steel spade 0 to10 cm of the sediment from the river bed. Each localisation was selected based on a preliminary study and each station was sampled at around ten points and then the samples were put together and homogenized to generate a representative sample of about 250g.Samples were transported and enveloped into aluminium foil (previously washed with methanol and dried in oven at 100°C). They were kept cool between 4 and 8°C under ice throughout transportation to the laboratory. All samples were transported in hermetic boxes then frozen at -20°C until analysis.

**Chemicals and reagents:** For this study, a group of 24 pesticides and PCBs including some of their transformation products were selected. These compounds are from different chemical families with a range of uses as well as different physicochemical characteristics and toxicity. Hexane and dichloromethane were of pesticide residue grade from Sigma-Aldrich. SPE cartridge used in this study were obtained from SUPELCO and deionized water was produced with an Arium Ultra-pure purification system, and a Fluka Anhydrous sodium sulphate was used to dry the samples. Standard solutions were

diluted from the stock solutions with hexane. Standard mixtures of pesticides were obtained from SUPELCO (TraceCERT) and PCBs from Sigma-Aldrich. The concentration of each component in the pesticide solution was 2000µg/mL and the PCB solution was about 10µg/mL. The analytes are listed in table 1. The surrogates standards (Tetrachloro-m-xylene and PCB29) from Sigma-Aldrich were used in our work.

**Table 1. Standard solutions analytes**

| **Pesticides** | |
|---|---|
| α-HCH (lindane) | Heptachlor |
| γ-HCH (lindane) | Aldrin |
| β-HCH (lindane) | Heptachlor epoxide |
| δ-HCH (lindane) | Tetrachloro-m-xylene (SI) |
| Dieldrin | Endosulfan Alpha |
| Endrin | Metoxychlor |
| Endosulfan Beta | p,p'-DDE |
| Endosulfan Sulfate | p,p'-DDD |
| Endrin Aldehyde | p,p'-DDT |
| Endrin Ketone | |
| **PCBs** | |
| PCB 29 (SI) | PCB 153 |
| PCB 28 | PCB 138 |
| PCB 52 | PCB 180 |
| PCB101 | |

**Sample preparation and instrumental analysis:** Sediment samples were lyophilized (Christ 2,4), ground, thoroughly mixed and then sieved through a 2mm diameter. Using the microwave oven, an amount of 10g from the sediment was placed in a teflon tube and spiked with surrogate standards. A mixtureof 40 mL of hexane/dichloromethane (50:50) was added.The oven was set at 1200 Watts, the temperature was programmed to reach 115°C in 10min and then isothermal at 115°C for 30 min. After extraction, the extracts were concentrated on a rotary evaporator to about 15mL. The temperature of the bath does not exceed 30°C. The extract was dried with anhydrous sodium sulphate. The dried extract was collected in the graduated tube and adjusted to 1mL by evaporating excess solvent under a gentle stream of clean dry nitrogen. Sulfur compounds and elementary sulfur such as mercaptans were removed in the process**.**

The main aim of the clean-up stage is to remove substances that could interfere with the final determination and quantitation of target analytes. Removal of interfering substances was accomplished by using copper (9, 28-30).Solid-phase extraction (SPE) is the dominant method for soil and sediment extracts purification (31, 32). A large number of sorbents are used for the isolation of organic compounds from the extract solutions, they include florisil, alumina, silica gel and many silica-based sorbents. For our case we used a solid phase extraction glass column (Upti-clean SPE glass columns $Na_2SO_4$/Florisil and a 12 ports glass vacuum manifold fitted with flow control valves to allow a fine adjustment of flow through the SPE column and disposal Teflon liners. Prior to the fractionation of the extract, SPE columns were rinsed with 10 mL of n-Hexane, the sample was applied on top of the SPE and the first fraction containing PCBs, pp'DDT and HCB is obtained by eluting with 10 mL of n-Hexane. The second fraction containing pp'DDD and Lindane is obtained by eluting 9 mL of n-Hexane: Dichloromethane (9:1) solution. The third fraction containing Dieldrin and Eldrin is obtained by eluting 7 mL of Dichloromethane.

**GC-MSD determination:** The chromatographic instrument was an Agilent 7890B with anAgilent 5977B simple quadrupole mass spectrometer. Using a MassHunter workstation software, data were processed for qualitative and quantitative analysis. The conditions used to determine the analytes are shown in table2 and the analytes parameters based on the method we used are in table 3.

**Table 2. Conditions used for the determination of analytes**

| | Injection conditions | Column type | Temperature program |
|---|---|---|---|
| OCPs | Splitless mode Heater: 240°C Pressure : 9,4 psi Septum Purge flow : 3mL/min | HP-5 MS 2 capillary column are used (15m x 0.25 µm x 0.25 µm)+ (15m x 0.25 µm x 0.25 µm) | 60°C for 2 min, increasing 20°C/min to 150°C, followed by 10°C/min to 200°C held for 20 min and increasing to to final 260°C held for 10 min Total run time was 28.5 min. |
| PCBs | Splitless mode Heater : 240°C Pressure : 10 psi Septum Purge flow : 3mL/min | | Start with 70°C, increasing 20°C/min to 150°C, followed by 10°C/min to 200°C held for 20 min and increasing to final 260°C held for 10 min Total run time was 19 min. |

**Table 3. GC-MS confirmation ions and retention time**

| Analytes | Ions 1 Quantification | Ion 2 Qualification | Retention Time |
|---|---|---|---|
| α-HCH (lindane) | 181 | 219 | 11.01 |
| γ-HCH (lindane) | 181 | 219 | 11.53 |
| β-HCH (lindane) | 181 | 219 | 11.62 |
| δ-HCH (lindane) | 181 | 219 | 12.16 |
| Heptachlor | 100 | 272 | 13.16 |
| Aldrin | 263 | 265 | 14.14 |
| Heptachlor epoxide | 353 | 81 | 15.59 |
| Tetrachloro-m-xylene (SI) | 207 | 219 | 16.82 |
| Endosulfan Alpha | 239 | 241 | 17.14 |
| p.p'-DDE | 318 | 246 | 18.42 |
| Dieldrin | 235 | 237 | 18.56 |
| Endrin | 263 | 81 | 19.88 |
| Endosulfan Beta | 239 | 241 | 20.66 |
| p.p'-DDD | 235 | 237 | 21.30 |
| Endrin Aldehyde | 249.9 | 345 | 22.10 |
| Endosulfan Sulfate | 272 | 387 | 23.45 |
| p.p'-DDT | 235 | 237 | 23.56 |
| Endrin Ketone | 281 | 207 | 25.42 |
| Metoxychlor | 227 | 228 | 26.10 |
| PCB 29 (SI) | 156 | 186 | 11.07 |
| PCB 28 | 256 | 186 | 11.39 |
| PCB 52 | 292 | 220 | 12.15 |
| PCB101 | 326 | 254 | 14.02 |
| PCB 153 | 360 | 290 | 15.73 |
| PCB 138 | 360 | 290 | 16.29 |
| PCB 180 | 394 | 324 | 17.83 |

**Quality assurance and quality control:** The method used for sediment samples was evaluated through recoveries, precision, linearity and sensitivity. The limits of detection and quantification of the method, both were calculated using spiked matrices. Recovery tests were carried out by spiking the sediments with 50ppb of surrogate standard. Two replicates were done in order to evaluate the precision of the method. Recoveries were higher than 65% for samples. Pesticide concentrations were validated against a comprehensive set of quality control parameters including laboratory and field blanks, matrix spikes and duplicate samples.

**Table 4. Comparative studies**

| Study area | ∑7OCPs | ∑3DDTs | ∑3HCHs | References |
|---|---|---|---|---|
| Mly Bousselham lagoon | 25.58 | 11.41–20.5 | 1.40 – 6.20 | *(Benbakhta et al., 2014)* |
| Sebou estuary, Morocco | 27.53 | 13.2 –25.4 | 3.78 – 9.10 | |
| Bouregreg estuary, Morocco | 13.17 | 2.4 –11.87 | 0.14– 6.2 | |
| Oum Rabi estuary, Morocco | 13.46 | 4.22 – 7.29 | 0.88 –6.3 | |
| Oualidia lagoon, Morocco | 22.86 | 7.49 - 23 | 2.54 –6.2 | |
| Port-of-Spain | 44.5–145 | 6.1–29 | 0.7–1.8 | A.Mohamed*et al., 2011* |
| Lake Qarun, Egypt | 1.01–164.8 | ND–5.88 | 0.13–100.6 | Barakat et al., 2013) |
| Pearl River Estuary, China | 5.53–38.21 | 0.88–24.45 | 1.74–28.78 | A. Qadeer et al., 2019 |
| Hooghly estuary, India | NA | 0.09 – 13.31 | 0.06 – 0.36 | S. Mitra et al., 2019 |
| Babitonga Bay, Brasil | NA | 0,18 - 122 | <DL – 1.2 | J. Rizzi et al., 2017 |
| Upper Sebou river, Morocco | 2.97 – 10.03 | 1.97 – 4.58 | 0.65 – 3.71 | Present study |

Blank contamination is the most common problem observed in the determination of pesticides at trace levels. Thus, precautions were taken to prevent contamination from personnel, organic solvents, equipment and glassware. Blank assays were performed employing an ultrapure water system, to check for laboratory background levels of the studied compounds. Thought the detected levels of the target compounds were low. In order to assure the quality of the results, field blanks were processed with the samples. It consists of deionized water put down in the same conditions than samples during sampling process. For each batch of 10 samples analysed including the water field blank, a procedural blank and a spiked recovery sample obtained by spiking at the low level, were routinely extracted and analysed under the same conditions as the ordinary samples.

# RESULTS AND DISCUSSION

**Occurrence and distribution of POPs in the studied area:** From the 24 targeted analytes, twelve components were found in all the locations trough the Sebou river (Figure 1). It is known that OCPs in the aquatic environment originate from the usage of pesticides in agriculture, farmland soil runoff inputs, and industrial wastewater discharge. HCHs isomers (α-HCH, β-HCH, γ-HCH, δ-HCH) and DDTs (p,p'-DDT, p,p'-DDE, p,p'-DDD) are two dominant pollutants of OCPs in the environment. ∑3HCHs concentrations ranging from 0.56 (S1) to 3.87 (S3) ppb and ∑3DDTs concentrations ranging from 0.96 (S1) to 5.30 (S5) ppb. For individual HCHs, α-HCH was the most dominant isomer with a maximum value of 2.31 ppb (S6), and p,p'-DDT was the most dominant with 2.61 ppb (S6). The same dominance was mentioned in other studies carried out at international level (33-36). The concentrations of ∑5PCBs in sediment samples were between 1.91 (S1)and 6.53(S4) ppb. The highest concentrations were found in (S4) (Sebou spring) and S5 (AllalFassi Dam). The lowest concentrations were found in S1, S2 and S3. The presence of higher concentrations of PCBs could be related to human actions and also to aerial transport of these pollutants (37, 38). Howeverburning, storage and deposition of PCBs-containing materials are the major sources of PCBs (39). It is known that PCBs are mainly transported by water and then quickly stored in the sediments because they are not very soluble in water(13, 40, 41). Levels of organochlorine pesticideswere higher downstream, especially in S5 and S6. These two stations are located in Allal El Fassi dam. It is known that the sedimentary compartment acts as a well where landfilling of contaminants is important and as a source that contributes, through its interface, exchange of organic contaminants between the water column and sediments (42).

Also several studies had highlighted that geography (e.g., elevation, terrain) and ecological factors can affect the transmission and distribution of OCPs from upstream to downstream (43, 44). The total organochlorine pesticides (OCPs) concentration in sediments varied from 2.97 (S1) to 10.03 (S5) ppb. Higher levels were found in Moroccan Atlantic coastin other studies and values of 27.53 ppb were found in Sebou estuary, 25.58 ppb in Moulay Bousselham lagoon and 22.86 ppb in Oualidia lagoon (45)

**Comparative analysis with other areas:** To further understand OCPs and PCBs pollution status in Sebou river, a comparative analysis was carried out with other areas in table 4. When compared with OCPs and PCBs occurrence in Morocco, ∑7 OCPs concentrations in upper Sebou river were lower than that reported from (45). When compared with foreign studies, ∑7OCPs concentrations were lower than some developed or developing countries, such as Egypt (46), and Spain (47).As for DDTs and HCHs compounds.

**Possible sources of OCPs and PCBs:** In order to define the origins and fates of HCHs and DDTs in the environment, we can use their isomer's ratios. Commonly the half-life period of alpha-hexachlorocyclohexane can be influenced by sedimentary properties. Both of α-HCH and γ-HCH can be transformed into beta-hexachlorocyclohexane because of its lower degradation possibility and vapor pressure (48).In consequence β-HCH have the ability to store easily in the sediments than other isomers (49, 50). Other scientists found that β-HCH was dominant in sediments of rivers or estuary area frequently (51).Like our study the concentrations of β-HCH were higher among the HCH congeners in most sampling sites with the exception of one site where α-HCH was dominant, reflecting the fact that HCHs majorly originated from historical inputs or long range transport. Some studies reported that the early usage of lindane was the dominant source to HCHs and mainly consisted of pure γ-HCH (52, 53).Based on different HCH isomer percentages in the industrial and agricultural fields, high ratios of α-HCH /γ-HCH (4.64-5.83) implied HCHs in sediments from industrial products, while low ratios of α-HCH /γ-HCH (<0.01) indicated that HCHs came from the agricultural application (50). In our study, the ratios of α-HCH/γ-HCH ranged from (0.81-4.94 ppb) which was in accordance with both industrial and agricultural residuals of HCHs. *p,p'*-DDE, *p,p'*-DDD, *p,p'*-DDT were identified in all sediment samples. *p,p'*-DDT and it's degradation products (*p,p'*-DDE, *p,p'*-DDD) contribute for a large part of total OCPs in the upper Sebou river. *p,p'*-DDT is converted by the microorganisms under aerobic conditions into *p,p'*-DDE and converted again to *p,p'*-DDD under anaerobic conditions (54, 35).*p,p'*-DDE degradation under

aerobic conditions may be reduced or inhibited in areas with high levels of DDT and significantly increased in areas with low concentrations. The ratio between DDE/DDT is used to indicate if the inputs are recent or old. A DDE/DDT ratio superior than 3 indicates historical inputs (53, 55).In our case, the levels of these two components shows a recent and old use of these molecules. Although no DDT is currently stockpiled in Morocco according to the national report on the management of persistent organic pollutants in Morocco (56), and the last stocks of DDT used for agriculture and public health, identified in 2010 have been disposed of in an environmentally sustainable manner.

**Conclusion**

This paper focused on the assessment of some organochlorine pesticides and some polychlorinated biphenyl in 3 years in six different locations covering an area of 50 km in the upperpart of Sebou river. This area was never studied before for these compounds and the results showed that for the 24 compounds targeted by the study only twelve compounds were detected in all the stations. The values of these contaminants do not exceed 12.27 ppb for the sum of 9 OCPs and 6.32 ppb for the sum of 5 PCBs, these levels are very low compared with international areas. The highest levels were detected at S9 and S10 both located on Allal El Fassi Dam testifying that due to the strong hydrophobic properties of POPs, sediments works as their natural sink keeping them in this way out of circulation from the system for a long time if not disturbed. Ratios between DDE/DDT and α-HCH/γ-HCH revealed that the contamination in this area is old and recent and from both industrial and agricultural use, even in the absence of industrial sites in the immediate vicinity of the study area.

**Conflicts of interest**

"There are no conflicts to declare".

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