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Écotoxicologie

CONTAMINATION OF SHELLFISH BY ORGANOCHLORINE PESTICIDES AND POLYCHLORINATED BIPHENYLS IN THE LAGOON OF SIDI MOUSSA (MOROCCO)

par

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This study concerns the analysis of Organochlorine Pesticides (OCPs) and Polychlorobiphenyls (PCBs) in samples of the European clam (*Ruditapes decussatus*), oyster (*Crassostrea gigas*) and common mussels (*Mytilus galloprovincialis*) collected from Sidi Moussa lagoon in July 2012, December 2013 and February 2013. A total of 13 OCPs and PCBs were targeted: εHCB, γ-HCH, DDT and its metabolites DDD and DDE, aldrin, heptachlor, PCB 28, PCB 52, PCB 101, PCB 138, PCB 153 and PCB 180. Assays were carried out by gas chromatography with an electron capture detector. The results show that organochlorine contamination was low and did not exceed tolerable levels according to European standards. The concentrations for ΣOCPs where higher than the concentrations for ΣPCBs. Oysters and mussels living in the water column showed higher concentrations than did the burrowing clam species. Aldrin showed the lowest concentrations compared to other chlorinated pesticides. Although the observed levels of contamination fall within current safety limits, careful future monitoring is recommended.

Keywords: Shellfish, Organochlorine pesticides, PCBs, Sidi Moussa lagoon, Morocco.

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Contamination des mollusques par les pesticides organochlorés et les polychlorobiphényles dans la lagune de Sidi Moussa (Maroc)

L'étude porte sur l'analyse des pesticides organochlorés et des polychlorobiphényles dans les échantillons de la palourde (Ruditapes decussatus), de l'huître (*Crassostrea gigas*) et des moules communes (*Mytilus galloprovincialis*) recueillies dans la lagune de Sidi Moussa en juillet 2012, décembre 2013 et février 2013.

Au total, 13 pesticides organochlorés (POCs) et PCB ont été ciblés: εHCB, γ-HCH, DDT et ses métabolites DDD et DDE, aldrine, heptachlore et PCBs (PCB 28, PCB 52, PCB 101, PCB 138, PCB 153 et PCB 180). Le dosage a été effectué par chromatographie en phase gazeuse avec un détecteur de capture d'électrons. Les résultats montrent que la contamination organochlorée était faible et ne dépassait pas les niveaux tolérables selon les normes européennes. Cependant, les concentrations les plus élevées étaient enregistrées pour les OCPs et avec les concentrations les plus faibles pour les PCBs. Les huîtres et les moules, vivant dans la colonne d'eau, présentaient des concentrations plus élevées que celles de la palourde. L'aldrine a montré les concentrations les plus faibles par rapport aux autres pesticides chlorés. L'étude indique que même si les niveaux actuels de contamination semblent se situer dans les limites de sécurité actuelle, une surveillance minutieuse est recommandée.

Mots-clés : Mollusque, Pesticides organochlorés, PCBs, Lagune de Sidi Moussa, Maroc.

Introduction

Whatever the origin of pollution, lagoon ecosystems are often the main sink for pollutants discharged directly or indirectly into the coastal marine environment. Lagoons attract attention not least because of their important aquaculture potential. Indeed, the production of seafood, an industry developed in Morocco since 1950, has flourished recently due to increasing demand in both domestic and international sectors. However, the large spatial and temporal variability of the environmental conditions in lagoons are conducive to concentrating pollution such that marine life, as well as seafood security, is threatened. An awareness of this risk is essential, since some organic pollutants can seriously impair both ecosystems and human health (TANABE & TATSUKAWA, 1994; UNEP, 1999). This has led many countries to make legal provisions for restricting or banning pesticide use.

Organochlorine pesticides and PCBs are major organic pollutants. These synthetic compounds have been widely used since the 1940s as pesticides in agriculture and various industrial applications, but they pose a risk due to their toxicity, high chemical stability and low biodegradability (KAMRIN, 1997; THOMPSON, 1999). They are highly lipophilic and toxic effects particularly occur following bioaccumulation through the food chain. Many of these compounds are recognized as endocrine modifiers. They are likely to interfere with the reproduction processes of animals, disrupt the immune system and attack the nervous system and organs such as the liver and the kidneys (YUKARI *et al.,* 2011; LANGER, 2010; ROOS *et al.,* 2012; LIU *et al.,* 2010). To assess the impact of these pollutants in the field requires knowledge of their presence and fate in various compartments of the environment.

The purpose of the present study is to evaluate the levels of contamination in commercially important molluscs in the Sidi Moussa Lagoon, Morocco. The results obtained are of relevance to policy makers and local authorities with respect to awareness about contamination levels and the activities generating polluting compounds. Such information should contribute to improving the sanitary quality of the lagoon ecosystem and protecting fishery resources in the surrounding area.

Materials and methods

Sidi Moussa Lagoon is situated on the Moroccan Atlantic coast, 36 km south of El Jadida (Figure 1). It is 5.5 km long and 500 m wide, with a total area of 4.2 km^2. The thermal regime of this coastal area reflects the influence of the ocean waters, having cool wet winters and relatively warm summers; annual average precipitation rarely exceeds 380 mm and the mean temperature is about 18.7°C (MAANAN, 2003). In the dry summer season, the trade winds from NNE to NE dominate. In the wet season, it is mainly the WSW and NW winds that dominate. The semi-diurnal tides range between 2 and 4 m. Tidal currents dominate intra-lagoon hydrodynamics. Sidi Moussa lagoon was classified as a SIBE (*Site d'Intérêt Biologique et Écologique*) and Ramsar site in 2005. The activities in the lagoon concern fishing, agriculture, salt harvesting in the saline upper lagoon and shellfish cultivation, these remaining the main commercial activity for the local population. The shellfishery concentrates on the clam, *Ruditapes deccusatus*.

Some important industrial units were developed at Jorf Lasfar, 15 km north of the lagoon, before protection of the coastline became a consideration (MAANAN, 2003). The natural lagoon area has significant ecological and ecotourism potentials, but it currently experiences serious environmental problems (EL KHALIDI *et al.*, 2011).

Since 2009 a monitoring strategy has been applied to areas of shellfish production in Sidi Moussa. Through a monitoring network for safety of the coastline, integrated actions involving multidisciplinary studies of the watershed and adjacent areas are now in operation.

For the present study, mussels and oysters were collected at four sampling stations (P1, P2, P3, P4) in July 2012, February 2013 and December 2013. Clams were collected only at P1 in summer and autumn 2012. The accumulations of organochlorine pesticides and PCBs in the tissues of Bivalvia were compared with those from natural populations of clams (*Ruditapes decussatus*) and caged populations of mussels (*Mytilus galloprovincialis*) and oysters (*Crassostrea gigas*).

The sampling strategy permitted the collection of a large number of individuals. Sixty specimens of each species were collected monthly, depurated in the laboratory, the soft part removed from shells and homogenized in pools of 60 individuals. Composite samples were stored at -20°C before freeze-drying and stored in glass jars away from light before analysis. Each composite sample of 5 g was spiked with an internal standard mixture: 2,4,5-trichlorobiphenyl and HCH. These standards were

used to calculate the efficiency of the overall recovery procedures. Samples were microwave extracted for 20 min in a 30 ml of mixture hexane/acetone (9:1, v/v). The residue was filtered through cotton glass and concentrated to a few ml, using a rotary evaporator in a bath (30°C). An aliquot was taken for treatment with concentrated sulphuric acid to destroy lipids. The clean up and fractionation was performed by passing the extract through a florisil (17 g) column that had been activated at 130°C for 12 h and partially deactivated with 0.5% of water. From this column, two fractions were extracted, the first fraction with 70 ml of hexane; the second with 50 ml of hexane/dichloromethane (70/30).

Each fraction was concentrated and injected into a GC (Hewlett Packard HP 6890) equipped with a 63Ni electron capture detector (ECD) and a split/splitless injector. The capillary column used was a DB5 (phenyl methyl siloxane, 30 m length, 0.32 mm ID, 0.52 µm film thickness).

The oven program was 60°C (2 min), 20°C min^{-1} to 150°C, and 2 min at 150°C, 10°C min^{-1} to 200°C, and 10 min at 200°C, 3°C min^{-1} to 260°C, and 15 min at 260°C. The temperature of the injector was maintained at 250 and that of the detector at 300°C. The carrier gas was helium, with a flow rate of 2 ml/min, and nitrogen as make-up gas, with a flow rate of 60 ml/min. Concentrations of HCBs, γ-HCH, DDT and its metabolites DDD and DDE, aldrin, heptachlor and PCBs (CB 28, PCB 52, PCB 101, PCB 138, PCB 153, PCB 180) were individually quantified by comparison with peak areas corresponding to the standard (BALLSCHMITER & ZELL, 1980).

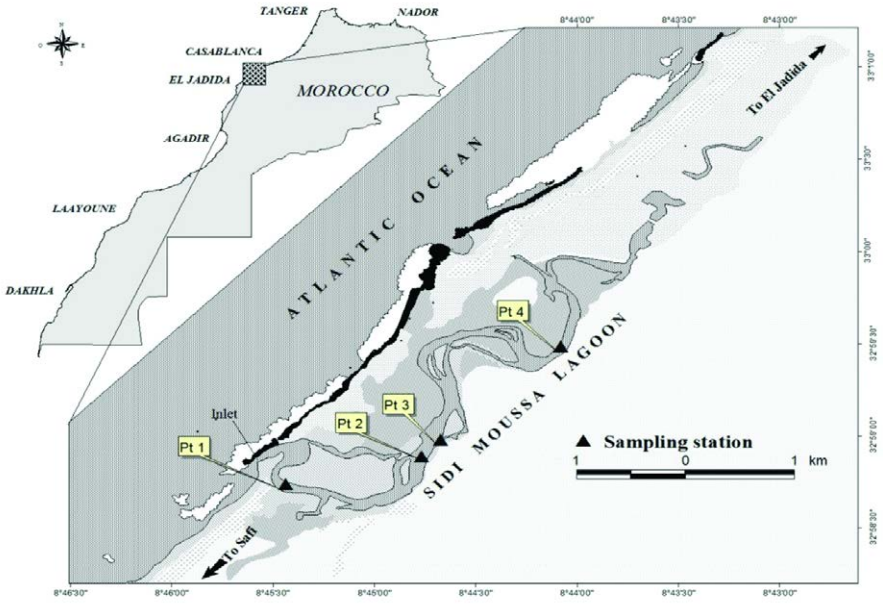

Figure 1
Location of Sidi Moussa Lagoon and the sampling stations.
Localisation de la lagune de Sidi Moussa et les stations d'échantillonnage.

Validation of analysis

In addition to blank analysis (representing less than 10% of the sample content) and in order to ensure the quality of the analysis, a double calibration was established at the level of the quantization of PCBs and organochlorine. The extraction yields were constant and above 70% over time.

The validity of this analytical method for chlorinated pesticides and PCBs was confirmed by the extraction and analysis of standard reference material IAEA 432 and 406, provided by the laboratory of the Environment of the International Atomic Energy Agency in Monaco, spiked with the same solution 2,4,5 TCB internal standards and εHCH that was used for doping samples, under the same chromatographic conditions. Hexane was injected as an injection blank between each injection of the sample to ensure the cleanliness of the GC injector. After injection, concentrations were calculated using the GC ChemStation Software version: G2090 BA Revision B 031.

Statistical study

XLSTAT software was used for all statistical analysis, the level of significance was set at 0.05, analysis of variance (ANOVA) was tested with the Fisher and Tukey tests, and correlations were tested by Pearson's analysis.

Results and discussion

In order to assess the levels of contamination, establish temporal variations and compare the contaminants levels between mussels, oysters and clams, the mean contents of ΣOCPs (HCB + lindane + aldrin + heptachlor + DDE + DDD + DDT) and ΣPCB (CB28 + CB52 + CB101 + CB153 + CB138 + CB180) were compared in three bivalve species (Figure 2). The levels of ΣOCPs are higher than those of ΣPCBs in all species, with a maximum of 19.1 ng/g dw in oysters in winter, followed by 15.9 ng/g dw in mussels and 9 ng/g dw in clams. The concentrations of ΣOCPs and ΣPCBs in oysters increased from summer to winter.

For the clams, the levels of ΣOCPs and ΣPCBs are lower than in oysters and mussels, with ΣOCP concentrations always higher than ΣPCB. The concentration profile is ΣOCPs > ΣPCBs. Although oysters tend to accumulate more organochlorines than other species, the ANOVA test shows that this difference is not significant (Table 1).

Table 1

ANOVA test results for interspecific variation.

Résultats du test ANOVA sur la variation interspécifique.

Contrast	Difference	Standardized difference	Critical Value	p-value > Diff	Significance
Mussels vs Clams	0.9465	1.7897	2.4445	0.1874	**No**
Mussels vs Oysters	0.0319	0.0490	2.4445	0.9987	**No**
Oysters vs Clams	0.9147	1.6913	2.4445	0.2223	**No**

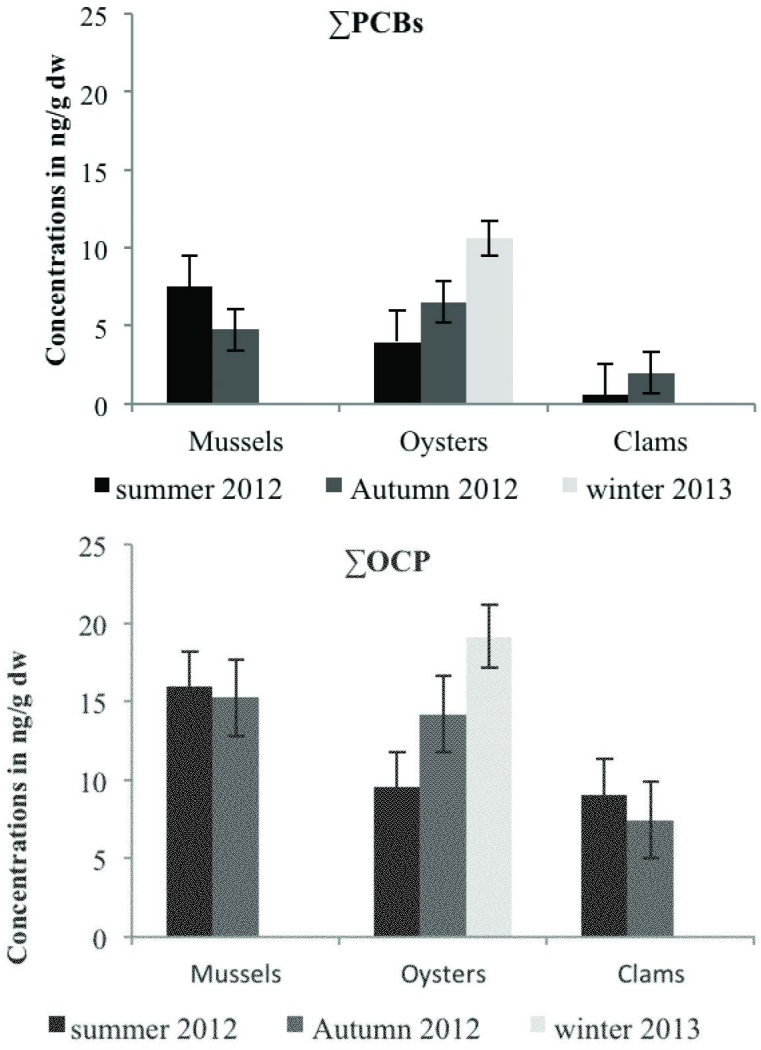

Figure 2
Seasonal variations in OCP and PCB contents (ng/g dw) in mussels, oysters and clams from Sidi Moussa Lagoon.
Variation saisonnière des teneurs en POCs et des PCBs (ng/g ps) chez les moules, les huîtres, et les palourdes de la lagune de Sidi Moussa.

The averages of the different sampling periods (Figure 3) show that12 of the organic compounds assayed are accumulated in fatty tissues of the three species of bivalves investigated, the exception being Aldrin. The levels of these compounds differ from one site to another. Maximum values for DDE do not exceed 7.43 ng/g dw in mussels and 7.84 ng/g dw in oysters. Concentrations of the other compounds are often < 5 ng/g dw in both mussels and oysters. Lindane and DDE are exceptions and

indicate an enrichment of P3 and P4 relative to P1 and P2 in mussels and oysters. However, these values remain within the permissible limits set by international bodies (UE regulation N° 1259/2011).

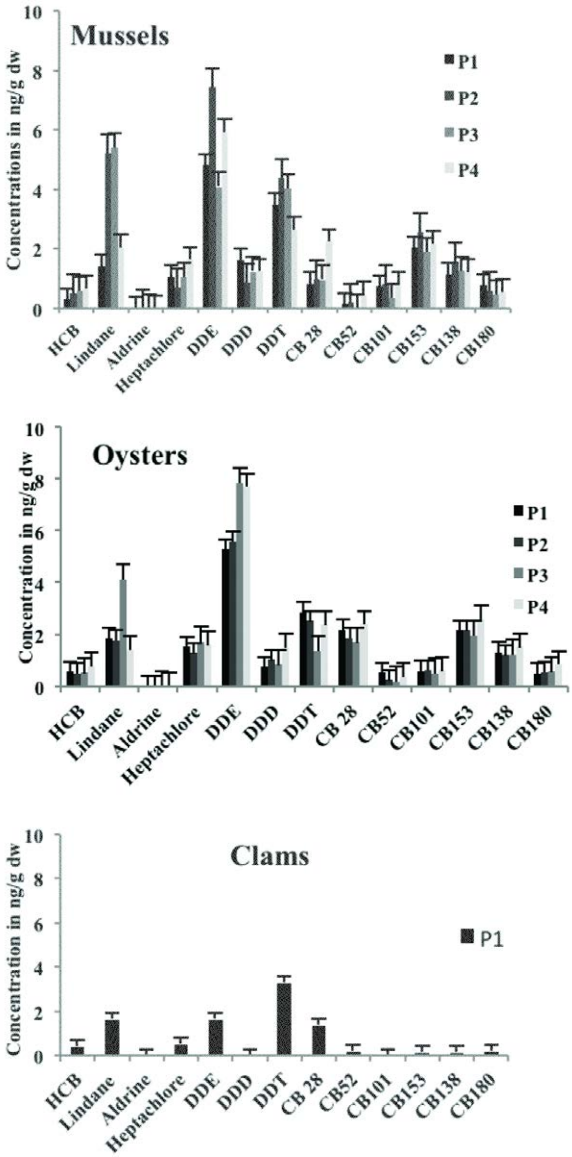

Figure 3

Spatial variations of OCPs and PCBs (ng/g dw) in mussels and oysters at four sampling sites, and in clams at one sampling site.

Spatial variations of OCPs and PCBs (ng/g dw) in mussels and oysters at four sampling sites, and in clams at one sampling site.

Table 2

ANOVA test results for spatial variation in organochlorines.
Résultats du test ANOVA sur la variation spatiale des composés organochlorés.

Contrast	Difference	Standardized difference	Critical value	p-value>Diff	Significance
P4 vs P2	0.3340	0.4986	2.6612	0.9590	No
P4 vs P1	0.2708	0.4084	2.6612	0.9767	No
P4 vs P3	0.0907	0.1138	2.6612	0.9995	No
P3 vs P2	0.2433	0.3418	2.6612	0.9861	No
P3 vs P1	0.1801	0.2553	2.6612	0.9941	No
P1 vs P2	0.0632	0.1134	2.6612	0.9995	No

The spatial variation in organochlorine concentrations was tested by analysis of variance and the differences were not significant (Table 2).

Figure 4 displays the proportions of the average content of DDT isomers in the different seasons at each point. These show a dominance of DDE concentrations. Low concentrations of DDT in mussels and oysters indicate the legacy from past use of DDT, an insecticide used against mosquitoes and crop pests in the Sidi Moussa agricultural area. The DDT isomers show the following concentration gradient in the Sidi Moussa lagoon: DDE > DDT > DDD.

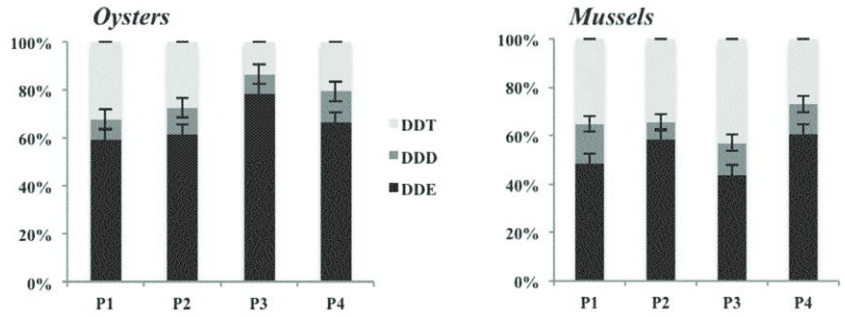

Figure 4

Relative percentages of DDT, DDE and DDD during the different seasons in mussels and oysters at four sampling sites in the lagoon.
Pourcentages relatifs du DDT, DDE et DDD dans les différentes saisons chez les moules et les huîtres.

These proportions show similarities in the accumulations in oysters and mussels at P2 and P3, especially for DDE (Figure 5).

As shown in Figure 6, the 153 CB is the largest component of the PCBs, followed by CB 28 with a maximum of 30%. The mussels and oysters showed similar profiles, dominated by CB153 and CB 28.

Regarding seasonal variation of organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) in three species, the results demonstrate that the sum of

OCPs is higher than that of PCBs. Intensive agricultural activities in green houses near the lagoon of Sidi Moussa are thought to be the main sources of these compounds.

The OCP results are similar to those reported by JAYED (2011) and JAYED *et al.* (2011a,b, 2015). DDE and lindane are most the commonly detected OCPs in the mussels and oysters, showing higher levels than for other compounds in the lagoon. These results are consistent with those obtained for the lagoon of Moulay Bousselham (BENBAKHTA *et al.*, 2007). The spatial variation in concentrations of OCPs and PCBs from downstream to upstream is related to the hydrology of the lagoon. As for PCBs, the metabolic mechanisms, contaminant inputs and nutritional behaviour of molluscs living in the lagoon could be responsible for its different distributions. The apportionment pattern demonstrated in the presents study is similar to results reported by JAYED (2011).

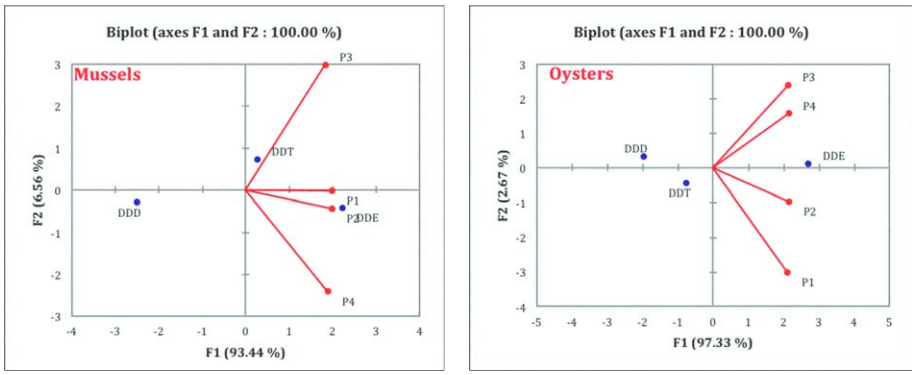

Figure 5

Correlation of relative percentages of DDT isomers in oysters and mussels for the four study sites.
Corrélation des pourcentages relatifs des isomères du DDT dans les huîtres et les moules des 4 sites.

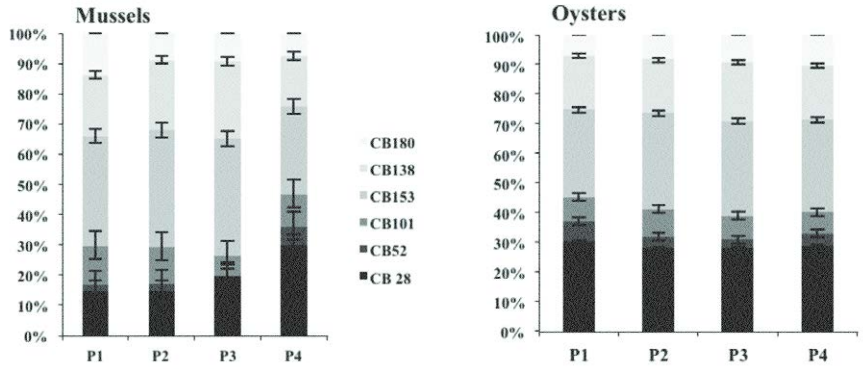

Figure 6

Relative percentages of PCBs congeners in mussels and oysters at the four sampling sites.
Pourcentages relatifs des congénères de PCB chez les moules et les huîtres dans les 4 points de prélèvements.

The comparison of the three species shows that the oyster has the highest content of OCPs and PCBs, followed by mussels and clams. Differences are probably related to the habitat preferences and feeding of these bivalves. Oysters and mussels are open water filter feeders (several litres per hour) and will therefore tend to concentrate more PCBs and OCPs than do clams. The latter are predominantly detritus filter feeders living in sediment and might filter less PCBs and OCPs some pesticides are retained by the sediment. More research on pesticide pathways in the lagoon is required to determine whether this is the case. It may also be that a difference in the lipid composition in the three bivalve species plays a key role, causing significant variations according to physiological and environmental conditions (GALINDO-BECT & FLORES-BAEZ, 1991).

Conclusion

The contamination of marine bivalves by organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) was assessed using material with two different behaviours, the mussels and oysters filter the water column and whereas the clam is a burrowing species. All three species showed higher levels of DDE and lindane than other organochlorine compounds; the PCB contents were all low, with the CB153 congener being present in the highest concentrations. The results indicate the impact on the lagoon of intensive activities in the agricultural sector, especially cash-crop production.

The levels of PCBs and OCPs are different in the three studied species, although the difference is not statistically significant. The same concentration gradient of OCPs is observed for PCBs.

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