

Persistent organic pollutants in *Mytilus galloprovincialis* mussel from El Jadida coast (Atlantic coast, Morocco)

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Abstract: Organic pollutants were measured in the soft tissue of *Mytilus galloprovincialis* by Gas Chromatography coupled with Electron Capture Detector (GC/ECD). The animals came from four stations along El Jadida coastline between January and December 2017. The qualitative analysis revealed the existence of the following compounds: aldrin, lindane, heptachlor and heptachlor epoxide. Heptachlor epoxide (HE) was quantified at all prospected stations. Levels ranged from 0.065 ± 0.02 µg/g d.w to 0.040 ± 0.01 µg/g d.w. Seasonal variation in the bioaccumulation of this compound has been observed. The highest concentrations are found in animals collected during late winter/early spring while the lowest ones are found during the summer season. The levels of HE detected in the present study remain within the international tolerance value reported by the Suisse DFI prescription in molluscs.

Keywords: *Mytilus galloprovincialis*, persistent organic pollutants, GC-ECD, heptachlor epoxide, Atlantic coast.

1. Introduction

El Jadida region has a strong agricultural potential coupled with a recent development of several industrial units. However, the residues of phytosanitary products used in intensive agriculture and the by-products of industrial activities release molecules that end up in different environmental compartments. [1, 2].

The *Mytilus galloprovincialis* mussel has been used in several global coastal biomonitoring programs [3, 4]. Indeed, the feeding mode of this filter-feeding bivalve and its wide geographical distribution make it an excellent pollution bio indicator. Our research team has been engaged in a local biomonitoring program of El Jadida (Atlantic coast) coastline for almost two decades. Researchers were first interested in trace metals levels in *M. galloprovincialis* [5, 6, 7] as well as in several annelid polychaete species [8], and then in their impact on physiological disturbances [9, 10, 11, 12]. The recent study on the growth of this bivalve from El Jadida littoral [13] has led us to evaluate other pollutants in the environment. Therefore, the objective of the present study is to assess persistent organic pollutants (POPs) at both qualitative and quantitative levels; using Gas Chromatography coupled with Electron Capture Detector (GC/ECD); in the soft tissue of *M. galloprovincialis* mussels from four stations of El Jadida shoreline.

2. Material and method

2.1 Study and sampling site

Mussels were collected at low tide, in the intertidal zone. Sampling was done at a monthly base for a period of one year (from January to December 2017) in four coastal stations of El Jadida "Fig. 1":

- i. Station H: Located 1 Km to the North of El Jadida (Coordinates 33°14'40.2''N 8°28'25.9''W).
- ii. Station J₁: Located 22 Km to the South of El Jadida (Coordinates 33°05'43.2''N 8°38'46.3''W).
- iii. Station J₂: Located 27 Km to the South of El Jadida (Coordinates 33°04'24.0''N 8°40'05.6''W).
- iv. Station J₃: Located 33 Km to the South of El Jadida (Coordinates 33°03'32.1''N 8°41'03.4''W).



Figure 1. Sampling stations (H, J₁, J₂, J₃) and study area of El Jadida coastline.

In each station, twenty adult mussels (3-5 cm size class) were collected and transported in glass containers with seawater. Once in the laboratory, the animals were cleaned and placed in oxygenated seawater for a purge period of 24 to 36 hours. Their flesh is then stored at -20 ° C until analysis.

2.2 Procedure

The experimental protocol is based on the use of gas chromatography technique coupled with Electron Capture Detector (GC / ECD) according to the UNEP / FAO / IOC / IAEA procedure [14]. The steps were as follow:

2.2.1 Extraction

The samples (10 g biota) are dehydrated at low temperature to remove residual water traces and are placed in glass tubes. A volume of 10 ml of n-hexane is added and then treated with ultrasound (BRANSON 1200 type, at 40 kHz and 100 W) for 45 minutes at ambient temperature. The emulsions formed are removed by centrifugation at 3500 rpm for 5 minutes.

2.2.2 Purification and Concentration

The purification is carried out using activated Florisil cartridge. The different stages are as follows: i) Conditioning which consists in impregnating the column with 4 ml of n-hexane for 5 min. ii) Introducing the sample drop by drop until the hexane level reaches the top. iii) The elution which is done by introducing a volume of n-hexane allowing the isolation and the separation of the desired compounds. The final solution is concentrated to a final volume of slightly less than 1 ml and then transferred to vials ready for injection.

2.2.3 Detection

The residues obtained are separated and quantified by gas chromatography (SHIMADZU 17-A) equipped with an Electron Capture Detector (63 Ni) and a non-polar capillary column [VARIAN MC; CP-SIL 8 CB 60 mx 0.25 mm x 0.25 µm (5% diphenyl, 95% dimethylpolisiloxane)]. The carrier gas is nitrogen. The injection is carried out in "splitless" mode and the injection volume is 1 µl. The temperature setting is 250 ° C for the detector and the injector. The column being on two temperature phases: from 60 ° C to 170 ° C (10 ° C / min) and from 170 ° C to 250 ° C (4 ° C / min).

The blanks received the same treatment as the biota samples. The standard solutions are provided by CHEM SERVICE and relate to the following compounds: hexachlorobenzene, endosulfan, DDT and its metabolites (DDT, DDD, DDE), Hexachlorocyclohexane and its isomers (αHCH and γHCH) and cyclopentadians (Aldrine, dieldrin, endrin, heptachlor and heptachlor epoxide). The pureness of the residues is around 99.5%. The identification of the compounds is based on the different retention times. The determination

of the compounds is based on external calibration technique. The compound concentration is obtained by comparison with the peak areas of the standard solutions.

2.3. Statistical data processing

The concentrations of chemical compounds are expressed as means \pm standard deviation. The comparison of the concentrations between the different study stations is made using Student's T statistical test.

3. Results

3.1 Qualitative scan

The determination of the compounds contained in the *M. galloprovincialis* mussel is based on the comparison of retention times between the samples analyzed and the standards. Figure 2a represents a typical chromatogram of the biota extract. Figure 2b is a blank chromatogram and figures 2c and 2d represent standard solutions chromatograms.

The blanks do not contain any detection peak, which means the absence of external contamination during various phases of sample handling.

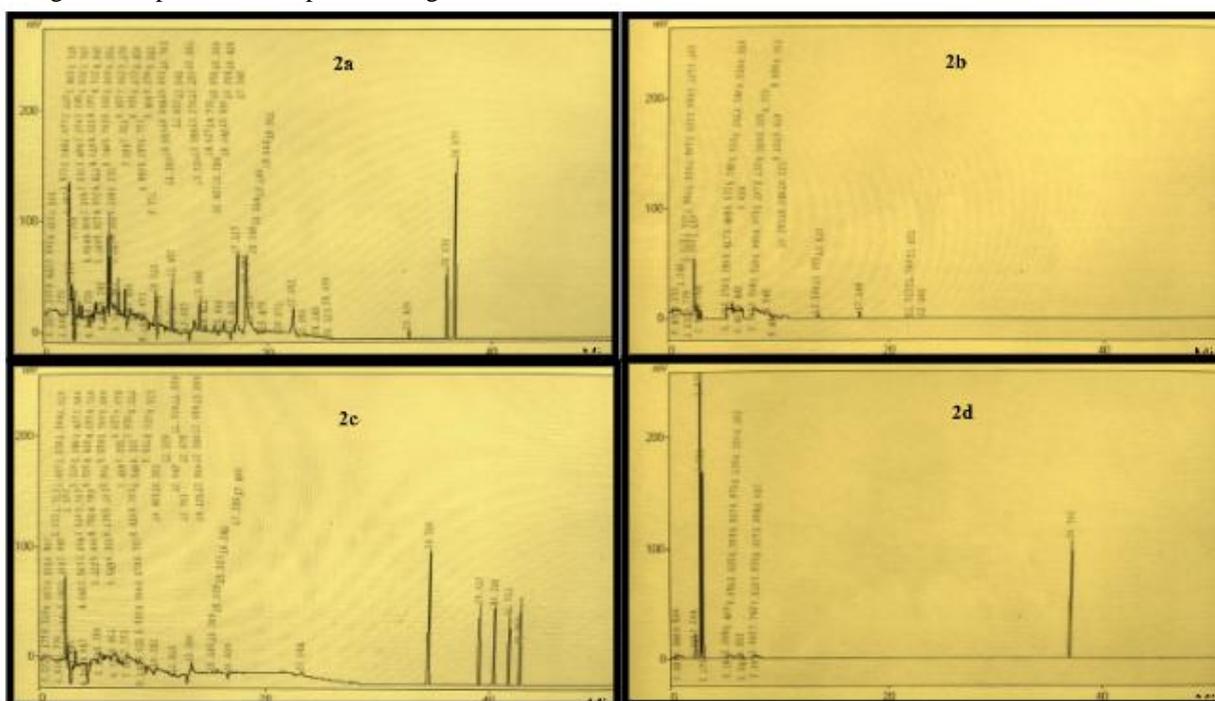


Figure 2. Chromatograms of *Mytilus galloprovincialis* biota extract (2a), blanks (2b) and standards (2c et 2d).

The whole chromatograms analysis, of the prospected stations, shows the presence of persistent organic pollutants. This contamination is characterized by a spatial variation (depending on the sampling station). The compounds found in the biota belong mainly to organochlorine pesticides and include: aldrin, lindane, heptachlor and heptachore epoxide. The different retention times indicating the maximum of each peak are recorded in Table 1.

The most common compound in prospected stations is heptachlor epoxide. The chemical formula of this strong contact pesticides is: $C_{10}H_5Cl_7O$ and which is a an oxidation product of heptachlor [15]. It has been added to prohibited substances of Stockholm Convention since 2001.

Table 1. Organochlorine compounds found in *Mytilus galloprovincialis* biota from El Jadida coastline.

Prospected stations	H	J ₁	J ₂	J ₃
Compounds	Heptachlor (35,986)	Heptachlor (35,986)	Heptachlor (35,986)	Heptachlor (35,986)
Retention time (Sec)	Heptachlor epoxide (36,669)	Heptachlor epoxide (36,669)	Heptachlor epoxide (36,669)	Heptachlor epoxide (36,669)
	-	Lindane (27,020)	-	-
	-	Aldrine (34,337)	-	-

3.2 Quantitative determination

The heptachlor epoxide standard's calibration curve is shown in Figure 3. The concentrations measured in the biota are based on the dry weight of mussel's soft tissue. The correlation coefficient (R^2) of the calibration curve is almost equal to 1 ($= 0.999$) and is above the quality threshold (greater than 0.995).

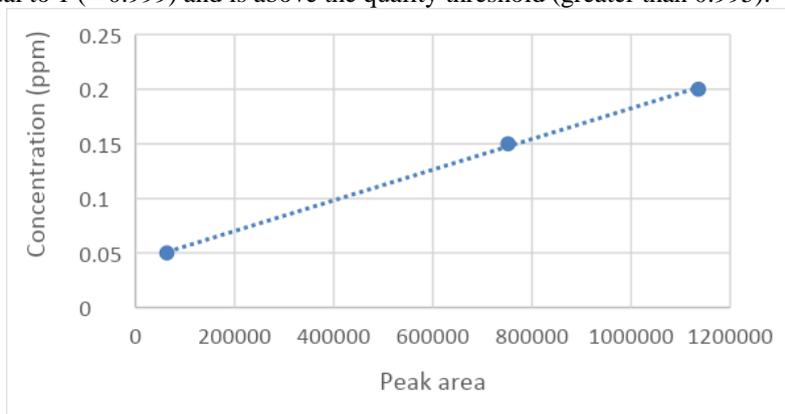


Figure 3. Calibration curve for heptachlor epoxide.

The highest levels of heptachlor epoxide are found in stations H and J₃. They are respectively $0.065 \pm 0.02 \mu\text{g} / \text{g d.w}$ and $0.057 \pm 0.008 \mu\text{g} / \text{g d.w}$ "Fig. 4". Mussels from stations J₁ and J₂ have relatively lower concentrations of respectively $0.05 \pm 0.02 \mu\text{g} / \text{g d.w}$ and $0.04 \pm 0.01 \mu\text{g} / \text{g d.w}$.

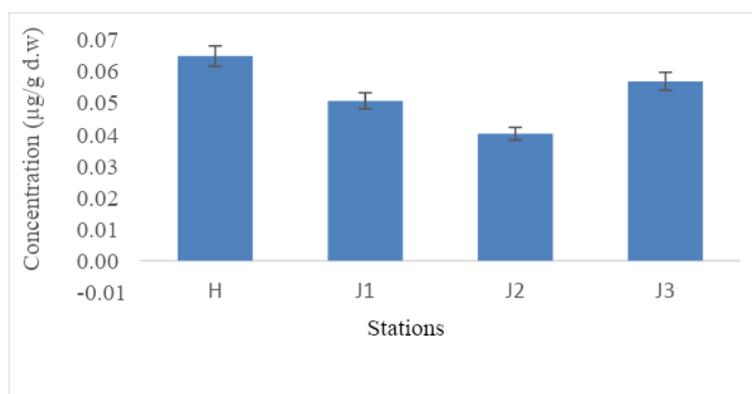


Figure 4. Spatial variation of heptachlor epoxide concentration in *Mytilus galloprovincialis* from prospected stations.

Statistical analysis of heptachlor epoxide concentrations reveals no significant differences between stations. These concentrations show a seasonal variation "Fig. 5". The highest levels are recorded during February and May in mussels from the majority of study stations: 0.103 $\mu\text{g} / \text{g d.w}$ in station H; 0.09 $\mu\text{g} / \text{g d.w}$ in J_1 ; 0.05 $\mu\text{g} / \text{g d.w}$ in J_2 and 0.074 $\mu\text{g} / \text{g d.w}$ in J_3 . The lowest levels are observed during August in mussels of station H and J_1 , with values lower than 0.02 $\mu\text{g} / \text{g d.w}$ and in the order of 0.029 and 0.048 $\mu\text{g} / \text{g d.w}$ respectively at J_2 and J_3 stations.

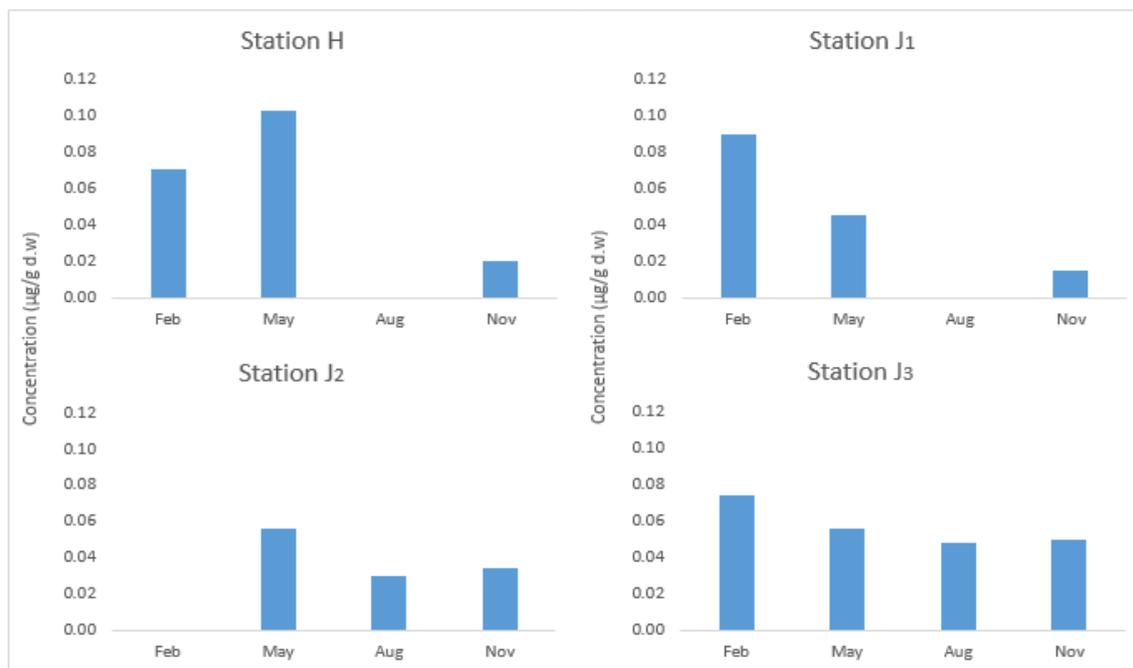


Figure 5. Seasonal variation of heptachlor epoxide concentration in *Mytilus galloprovincialis* from prospected stations.

4. Discussion

The biota chromatogram analysis using Gas Chromatography coupled with an Electron Capture Detector (GC / ECD) allowed us to identify organochlorine pesticides presence in *Mytilus galloprovincialis* soft tissue collected along the coast of El Jadida. The animals at stations H, J_2 and J_3 are contaminated with heptachlor and heptachlor epoxide, while those of station J_1 contain, in addition, aldrin and lindane. The quantitative analysis concern heptachlor epoxide; the most common compound to all prospected stations. Levels vary between $0.065 \pm 0.02 \mu\text{g} / \text{g d.w}$ and $0.040 \pm 0.01 \mu\text{g} / \text{g d.w}$; depending on the season. The lowest levels recorded in summer and the highest in late winter and early spring.

Numerous studies have reported the preferential accumulation of certain PCBs in the tissues of marine organisms [16, 17, 18]. The seasonal variation in bioaccumulation of PCBs in molluscs has been noted by several authors [19, 20]. The concentrations recorded in the present study remain far below or similar to those found in previous works. In *Mytilus galloprovincialis* from Bizert lake (Tunisia), they recorded a concentration of 0.26 $\mu\text{g} / \text{g d.w}$ [19]. At the Asian Pacific coasts, the bio-monitoring program initiated detects PCB concentrations around 7.4 and 84 $\text{ng} / \text{g d.w}$ in *M. galloprovincialis* and around 0.20 and 3.4 $\text{ng} / \text{g d.w}$ in *M. edulis* [3]. In Moulay Bouselham lagoon (Morocco), organochlorine pesticides residues were analyzed by GC / ECD [21] and the concentrations of heptachlor epoxide (HE) remain far below those recorded in the present study; 0.2 $\text{ng} / \text{g d.w}$ in clam individuals, 0.31 $\text{ng} / \text{g d.w}$ in mullet fish and no trace detected in *M. edulis*.

5. Conclusion

The levels of organochlorine compounds found in *Mytilus galloprovincialis* soft tissue from different stations on El Jadida coastline remain similar to those found by scientists in pacific coast bio-monitoring program. The heptachlor epoxide concentration recorded are close to the international tolerance value reported by the Suisse order DFI on the maximum limits applicable to pesticide residues present in or on vegetables or animal products; which is 0.05 $\text{mg} / \text{kg d.w}$ in mollusks [22].

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