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Rice fields regulate organochlorine pesticides and PCBs in lagoons of the Nature Reserve of Camargue

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ABSTRACT

In order to assess pollutant transfer in Camargue ponds from bordering agrosystems, a biomonitoring assay was conducted in irrigation and drainage channels of rice fields in the Rhone Delta (France). A filter-feeding bivalve, the Asian clam, *Corbicula fluminea*, was used as bioindicator and caged in upstream and downstream channels of an area of conventional rice fields. After 6 weeks incubation, many lipophilic biocides were identified in *Corbicula* tissues, including pesticides used in rice plantations (pretilachlor, oxadiazon), pesticides presumed in use in the Rhone basin [diuron and its metabolite 3,4 dichloroaniline (3,4-DCA)] and organochlorine pesticides (OCPs) banned for several decades. In addition, PCBs were highly bioaccumulated in *Corbicula*. Downstream bivalves had significantly lower concentrations of OCPs, PCB and 3,4-DCA. However, the exposure biomarkers (glutathione S-transferase, catalase and propionylcholinesterase) were not correlated with the decreased concentrations. The results of this experiment raise several questions concerning the potential role of immersed plants in a retention process.

1. Introduction

In 2007, Babut and Miege revealed a significant polychlorinated biphenyl (PCB) contamination of fish populations in French rivers, especially in the Rhone River. A French governmental plan was developed, with as objectives the improvement of scientific knowledge on the outcome of PCBs in aquatic environments and the management of such pollution. PCBs are ubiquitous, persistent, lipophilic, bioaccumulative and toxic, even highly toxic, microcontaminants (Falandysz et al., 2002). They were extensively used in industry, and though banned for decades, are still found in the majority of river sediments and accumulate in the food chain. Like PCBs, organochlorine pesticides biomagnify along aquatic trophic webs (Falandysz et al., 2004; Lanfranchi et al., 2006). Various depollution techniques have been put forward, with particular emphasis on methods which respect the equilibrium of the ecosystem, for example the promising development of phytoremediation technologies (Williams, 2002; Suresh and Ravishankar, 2004; Smith et al., 2007; Schroder et al., 2008). Plants were able to transform, degrade, accumulate, volatilize and extract PCBs (Mackova et al., 2006). In this context, cultivated immersed plants may provide a motivating opportunity even though little is known about the impact of organic contaminations on cultures irrigated with water from the Rhône River.

The Reserve of Camargue (43°N; 4°E; France), located in the centre of the Rhone delta (193 000 ha), between the two main arms of the river, was designated in 1977 by the International co-ordinating council of the Man and the Biosphere (MAB) programme as a Biosphere Reserve (revised in 2006). In its centre is the Nature Reserve of Camargue (NRC) containing interconnected marshes and lagoons, of which the largest is the Vaccarès Lagoon (6500 ha) (Chauvelon et al., 2003). The Vaccarès Lagoon is separated from the Rhône and its main inflow consists on water from channels that discharge the runoff of surrounding fields (Chauvelon, 1998). Thus, in spite of multiple statutes of protection, the Vaccarès Lagoon is exposed to various anthropogenic disturbances, among them, effluents from rice growing requiring large volumes of water drained from the Rhone River. This water contains agrochemical products, such as pesticides and PCBs coming from the Rhone Basin, in addition to the chemical compounds used in the rice fields (Comoretto et al., 2007, 2008). In various studies published over the last decade, we have analyzed contamination in biota of the Vaccarès Lagoon. We have demonstrated that this ecosystem is exposed to a wide range of organic contaminants (pesticides, agricultural inputs, industrial products, hydrocarbons, etc.) and that organisms located at the top of the trophic web, e.g. eels, develop more or less reversible lesions and necrosis, potentially related to this contamination (Roche et al., 2000, 2002a,b; Oliveira Ribeiro et al., 2005, 2008; Buet et al., 2006).

In Camargue, rice growing is achieved by immersion, and uses a large variety of chemicals (Gamon et al., 2003). Recently, chemists

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showed that 90% of currently used pesticides found in the water of lagoons and channels resulted from rice growing (Comoretto et al., 2007, 2008). Further to this assertion, our aim was to know which organochlorine pollutants were coming from the rice fields and others from the Rhone River.

We thus planned a biomonitoring program designed to assess the nature and the quantity of lipophilic pollutants flowing into the Camargue ponds via the drainage channels of adjacent rice fields. A filter-feeding bivalve, the Asian clam, *Corbicula fluminea*, was chosen as bioindicator to be caged in channels upstream and downstream from a conventional rice field area. *C. fluminea* live at the sediment–water interface and are know to accumulate chemical contaminants, notably pesticides (Galloway et al., 2002), PCBs (Peterson et al., 1994; Colombo et al.,1995, 1997), polyaromatic hydrocarbons (Narbonne et al., 1999; Zohair et al., 2006) and heavy metals (Baudrimont et al., 1997). Biomonitoring studies have also shown the high biomarker ability of enzymatic parameters in the Asian clam (Mora et al., 1999; Vidal et al., 2001,2002a,b; Cooper and Bidwell, 2006). In addition, the use of encaged filterer bivalves transplanted from unpolluted area, offer a valuable means for detecting disturbances of natural environments (Cossu et al., 1997), notably *C. fluminea* (Andres et al., 1999; Baudrimont et al., 1999).

Multiple xenobiotic screening and assessment of enzymatic biomarkers were thus conducted concomitantly with *C. fluminea*. The purpose of this study was to estimate the bioaccumulation of organochlorine pesticides and PCBs and to evaluate the responses of several *C. fluminea* enzymatic biomarkers: glutathione S-transferase (GST), catalase (CAT) and propionylcholinesterase (PChE).

2. Material and methods

2.1. Study area

The Biosphere Reserve of Camargue, located within the Rhone delta in Southern France, is the largest coastal wetland of Western Europe. The study sites were positioned inside the buffer zone of the Reserve which corresponds to the Fumemorte basin, i.e. about half of the catchment area of the Vaccarès system. In this area, numerous soils are devoted to growing rice (Fig. 1). The first station (1) was in the channel named 'Aube de Bouic' which, with 30.10^6 m^3 drained between 1997 and 2000, constitutes the third leading water contribution of the basin. The water is collected from the Rhone River, downstream from the city of Arles, then irrigates rice field parcels and is drained via the Fumemorte Channel (2) to the Vaccarès Lagoon. The physicochemical parameters of water in stations 1 and 2 (upstream and downstream from the rice fields, respectively) were monitored throughout the experimentation.

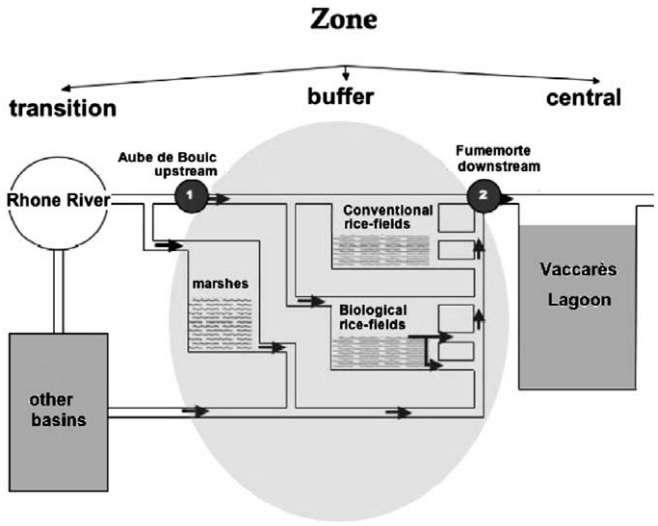

Fig. 1. Schematic representation of the caging experiment. 1: upstream and 2: downstream from conventional rice fields in the Rhone Delta. Three cages by site. Six *Corbicula* were sampled in each cage for the biomonitoring test.

2.2. Bivalves collection

A total of 108 individuals of Asian clams, *C. fluminea,* were hand-collected in a small channel 'Rigole de Méjane' located at the Northwest of the Rhone Delta and represents a hydrological unit (Comoretto et al., 2008).

A homogeneous sampling of 18 individuals was distributed in six cages ($40 \times 28 \times 20$ cm). Morphometric parameters were measured at the beginning of the caging – weight (11.1 ± 0.6 g), length (33.3 ± 1.0 cm), width (30 ± 1 cm), height (19.7 ± 0.4 cm) of the shells – and at the end in order to evaluate their condition indexes. The analysis of organochlorine and metallic contaminants and biomarkers was carried out on six individuals sampled randomly in each cage, i.e. bioaccumulation and biomarker measures were carried in a sample of 18 individuals. All *Corbicula* were alive after 6 weeks-exposure. The assessment of metal contamination will be published next.

2.3. Experimental setup

After 7 d acclimatization, three cages were placed on the channel sediment for 6 weeks (from June 25 to July 29, 2006) upstream and downstream from parcels of conventional rice fields. The acclimatization process is required to the clean-up of lipophilic molecules and homogenization of sampling. Moreover, to avoid the intrinsic or extrinsic effects related to the *Corbicula* enclosure (i.e. the cage-effect) – like nature of the cage, current environmental factors or other likely stressing agents – the principle of multi-replicates was applied. Three cages, with the same number of individuals from the same population, were placed in the two experimental sites. As the condition index and the body fat are the biometric parameters potentially affecting the intensity of the bioaccumulation of pollutants lipophilic, we verified their lack of inter-individual variability. The physicochemical parameters and the primary production (chlorophyll a) were checked throughout the experiment. Dissolved oxygen, temperature (oxymeter HACH, LDO HQ10), conductivity (conductimeter WTW, Cond 340i), ammonium NH_4^+, nitrite NO_2^- and nitrate NO_3^- (electronic spectrophotometers HANNA instruments) were recorded daily (at 10 and 18 h) during the first 2 weeks, then twice a week until the end of the experiment. Chlorophyll *a* and pheopigment contents were determined according to the standardized protocol AFNOR T90-117. (AFNOR, 1994). Total Suspended Particulate Matter (SPM) and Volatile Particulate Matter (VPM) were estimated according to method AFNOR T90-105 (AFNOR, 1994).

2.4. Biometric parameters

Height, length and width of shells were measured using an electronic slide caliper. Moisture content of soft tissue was estimated gravimetrically after drying of 0.2 g soft tissue at 105 °C for 24 h. The condition index was estimated according to the French Association for Standardization NF V45056, (AFNOR, 1994) *CI* = (drained weight of soft tissues/total weight).

2.5. Chemical analyses

The extraction of lipids and lipophilic compounds was then performed using an accelerated solvent extraction (ASE200) System

(Dionex, Voisins le Bretonneux, France). Soft tissue of Asian clams was dissected, then homogenized and mixed with clean Fontainebleau sand (1:3 w/w) and 1 g hydromatrix. The mixture was introduced into a 33 ml ASE cell and the extraction was then performed using dichloromethane: methanol (2:1 v/v) as solvent and under the conditions described by Toschi et al. (2003) – temperature: 120 °C; pressure: 100 bar; heat time; 6 min; static time: 10 min; flush volume: 60%; purge time: 120 s; two cycles. The final volume was evaporated using a rotary evaporator (Buchi). Lipid amounts were gravimetrically determined, then 2 mL hexane was added to the crude extract (CE) and 100 μl of dicofol solution in hexane (0.5 ng μL^{-1}) was introduced as external standard. The extract was subsequently purified by solid phase extraction (SPE) on florisil (MgO_3Si), following the EPA method 3620 (Bond Elut Florisil, 1 g, 200 μM particle size, Varian, Les Ulis France), first with hexane, to eluate ∑DDT, HCB and PCB, then with hexane:diethylether (90:10 v/v) for OCP clean-up. The eluent was carefully evaporated to dryness under a nitrogen stream and finally brought up to 500 μl with hexane for analysis.

Organochlorine compounds were analyzed by gas chromatography with a Clarus 500 (Perkin–Elmer), using ECD (electron capture detection) with a ^{63}Ni Source and nitrogen as make-up gas according to an adapted procedure of the EPA Method 8081a, previously described (Oliveira Ribeiro et al., 2005, 2008). Separation of compounds were achieved using a 30 m column, internal diameter 0.25 mm, PE5 fused silica column (PerkinElmer, Courtaboeuf, France) and ultra-high purity nitrogen (99.9999%) (Alphagaz N_2-2, Air Liquide, Grigny, France) as carrier gas. The injector and detector temperatures were 280 and 350 °C, respectively. For the OCPs, [α-, β-, γ-, δ- HCH; hexachlorobenzene (HCB) ; aldrin; dieldrin; α-, β-endosulfan and endosulfan sulphate; heptachlore and heptachlore epoxide cis; endrin and endrin aldehyde; fipronil ; oxadiazon; pretilachlore; diuron and dichloroaniline], the initial GC oven temperature was 200 °C (12 min hold) followed by an increase to 210 °C at 10 °C/min (30 min hold) and a rapid increase to 260 °C at 40 °C/min (3 min hold). For PCBs, HCB and DDT (*op′*-DDE, *pp′*-DDE, *pp′*-DDD, *op′*-DDT, *pp′*-DDT), the GC conditions were: after an initial temperature 140 °C (12 min hold), the oven was ramped at 40 °C/min to 170 °C (19 min hold), then at 40 °C/min to 200 °C (25 min hold) and finally at 45 °C/min to 270 °C (4 min). Among the 209 PCB congeners, seven compounds considered as indicator PCBs (IUPAC no 28, 52, 101, 118, 138, 153, 180); 12 dioxin-like PCBs (IUPAC no 77, 81, 105, 114, 118, 123, 126, 156/157, 167, 169, 170) and 9 others (IUPAC no 8, 18, 31, 44, 70, 151, 128, 195, 194) were investigated. All reference materials were produced by the ISO9001 certified laboratories of Dr. Ehrenstorfer as part of the Reference Standards Program provided by the Society CIL Cluzeau, (F33220 Sainte Foy la Grande, France). Under the specified conditions, the detection limit ranged from 0.05 to 0.20 ng g^{-1} in Asian clam tissues (dry matter normalized data). The analyses were performed in the Department of Ecology, Systematic and Evolution in Paris 11 University (France).

2.6. Biomarker analysis

The soft tissue was dissected, rinsed in a phosphate buffer (100 mM K_2HPO_4/KH_2PO_4, pH 7.4), weighed then homogenized (1:3 w/v) using an Ultra-Turrax T25 (Janke-Kunkel). The homogenate was centrifuged at 9000 g, 30 min, 4 °C (J2-MC Beckman). The supernatant (S9) was used for biomarker determinations in which the protein content was estimated by the Bradford method (1976).

GST activity in cytosol was assessed by monitoring the conjugation of GSH with 1-chloro-2,4-dinitrobenzene (CDNB) according to Habig et al. (1974). Catalase activity was evaluated using the hydrogen peroxide breakdown method (Aebi, 1984). The method described by Mora et al. (1999) adapted from the method of Ellman et al. (1961) was used to measure cholinesterase activity, with propionylthiocholine as substrate (propionylthiocholine esterase, PChE). All enzyme activities were kinetically measured, with at least two replicates.

2.7. Data analysis

Inter-site variations were analyzed using ANOVA, followed by the Scheffe and Bonferroni–Dunnett post-hoc tests. To compare bioaccumulation levels and enzyme activities, a 5% significance level was used.

3. Results

3.1. Abiotic data

NO_2^- concentrations, ranked from 0.13 mg L^{-1} in the irrigation channel to 0.43 mg L^{-1} in the drainage channel at time 0, were decreasing during the experiment. NO_3^- showed an inverse profile, about 1.11 downstream and up to 10.5 upstream at the end of the experiment. NH_4^+ concentrations were inconsistent, lower upstream (0.07 ± 0.06 mg L^{-1}) than downstream (0.82 ± 0.50 mg L^{-1}).

The temperature of the water increased during the experiment from 23.7–25 to 26.4 °C with a day-to-day amplitude variation of about 1.6 °C upstream and 2.8 °C downstream. In each site, oxygen saturation was stable throughout the experiment, i.e. 80.0 ± 1.4% and 44.4 ± 4.1% upstream and downstream from the rice fields, with a diurnal variation of 10.6 ± 2.1% in the irrigation channel and 26.5 ± 4.2% in the drainage channel. Whereas the salinity was low and stable, 0.0‰ and 0.1‰, respectively, conductivity was higher and more inconsistent in the downstream channel, 679 ± 59.6 μS cm^{-1}, than in the upstream channel, 384 ± 10.4 μS cm^{-1}. Chlorophyll *a* and pheopigment contents were four times higher downstream than upstream, (12.1 ± 1.2 μg L^{-1} vs 4.3 ± 1 μg L^{-1} and 14.2 ± 2.0 μg L^{-1} vs 3.4 ± 2.2 μg L^{-1}, respectively). Total SPM and VPM showed no significant inter-site variations (SPM: 48 ± 5.5 and 59.7 ± 4.7 mg L^{-1}; VPM: 7.7 ± 0.7 and 11.2 ± 0.7 mg L^{-1}, upstream and downstream from rice fields, respectively).

3.2. Morphometric parameters

For each treatment, 18 individuals from three cages (6 × 3) were analyzed in order to avoid any 'cage-effect', i.e. change due to specific enclosure conditions (Fig. 1).

After a 6-week experiment, the weight gain varied significantly according to the site of incubation (Table 1). *Corbicula* caged in the drainage channel (downstream) showed higher weight, better growth, and enhanced condition index; on the contrary, the lipid level did not differ by cage location, whereas soft tissue hydration was higher in downstream clams.

Table 1
Biological parameters of *Corbicula* after 6 weeks caging upstream and downstream from conventional rice fields in the Rhone delta. Mean ± standard error; number of individuals = 18; T_0 and T_{6w}: data at experiment beginning and end (6 weeks), respectively.

	Upstream	Downstream	*P*-Value
Total weight T_0 (g)	11.1 ± 0.6	11.1 ± 0.6	
Total weight T_{6w} (g)	11.3 ± 0.5	11.9 ± 0.7	0.003
Soft tissue weight T_{6w} (g)	1.59 ± 0.05	2.43 ± 0.07	<0.0001
Weight gain (%)	1.8 ± 0.4	7.2 ± 0.9	<0.001
Moisture (%)	81.3 ± 0.7	86.6 ± 0.3	<0.0001
Condition index	14.1 ± 1.6	20.4 ± 2.5	0.001
Total lipids (mg g^{-1}) dry weight	95.5 ± 3.6	121.6 ± 12.1	0.07

3.3. Pesticide bioaccumulation

In *Corbicula* caged both upstream and downstream from rice fields, pesticide bioaccumulation was greatest for diuron[3-(3,4-dichlorophenyl)-1,1-dimethylurea] and its main biotransformation product, 3,4-dichloroaniline (3,4-DCA). Diuron content (≈1600 ng g^{-1} dw) did not show significant variation, whereas 3,4-DCA concentration upstream the rice plantations was, on average, twice than in *Corbicula* immersed downstream rice fields ($p = 0.09$; 6473 ± 1885 ng g^{-1} dw and 2634 ± 884 ng g^{-1} dw from upstream and downstream cages, respectively).

Corbicula from the two sites strongly accumulated oxadiazon [79.5–2154 ng g^{-1} dw], with higher concentrations in bivalves caged in the drainage channel ($p = 0.021$) compared to those from the irrigation channel (Fig. 2). On the contrary, pretilachlor content was low, notably in downstream *Corbicula* (i.e. [nd-718 ng g^{-1} dw] vs [nd-75.7 ng g^{-1} dw]). Fipronil was not detected in bivalves sampled downstream and was low in the others.

Most of investigated OCPs were more concentrated in *Corbicula* from upstream rather than from downstream channels (Fig. 2). Numerous banned pesticides [lindane, heptachlor, endosulfan, dieldrin, hexachlorobenzene (HCB) and DDT] and their metabolites (or isomers) were detected. Ranked by concentration levels, they were lindane > ∑DDT > dieldrin > ∑heptachlor > ∑endosulfan > HCB. *Corbicula* were highly contaminated with lindane (γ-HCH) (86% of ∑HCH): 291 ± 40 and 176 ± 9.7 ng g^{-1} dw upstream and downstream, respectively, showing a significant inter-site difference ($p = 0.016$). A similar difference was found for DDT and its metabolites ($p < 0.001$, 207 ± 18.4 ng g^{-1} dw vs 56.0 ± 8.9 ng g^{-1} dw, upstream and downstream respectively) as well as ∑endosulfan ($p = 0.003$, 124 ± 20 ng g^{-1} dw vs 47.0 ± 6.4 ng g^{-1} dw) and ∑heptachlor ($p = 0.0005$; 153 ± 19 ng g^{-1} dw vs 68.2 ± 7.4 ng g^{-1} dw). Dieldrin was found at a higher concentration in upstream clams ($p = 0.031$; 190.4 ± 53.5 ng g^{-1} dw vs 55.2 ± 12.1 ng g^{-1} dw).

Among the identified molecules, only endrin and aldrin did not show any variation, but their concentration was low, about 2 and 30 ng g^{-1} dw, respectively. In addition, numerous peaks emerging on chromatograms were unknown, but usually, their areas were greater in extracts from upstream *Corbicula*.

3.4. PCB bioaccumulation

Indicator PCBs were highly concentrated in upstream *Corbicula* (Table 2). Ranked according to descending concentrations, compounds found were CB153 > CB8 > CB52 > CB118 > CB138 > CB101 > CB123 > CB180. Moreover, concentration levels in downstream *Corbicula* of these congeners declined greatly (−59.0% ± 0.2). Following the accumulation levels, their classification became CB153 > CB52 > CB70 > CB118 > CB28 > CB44 > CB101 > CB138. Regardless of their toxic potential, this decrease in concentration was also observed for compounds with the highest toxic equivalent, [who-TEQ, (Van den Berg et al., 1998)], the dioxin-like PCB126 and PCB 169%, −73% and −46%, respectively.

Generally, bivalves caged upstream from the rice fields were significantly more burdened with highly chlorinated PCB congeners (hexa- hepta- octa-chlorobiphenyls). Here these high LogK_{ow}, lipophilic, biomagnifiable and minimally degraded molecules accounted for 61.5 ± 3.3% of upstream and 46.6 ± 2.8% of downstream ∑PCBs. In addition, Fig. 3 shows that the difference of PCB with LogK_{ow} > 6.5, was significantly stronger than disappearance of lighter molecules, suggesting a dechlorination process.

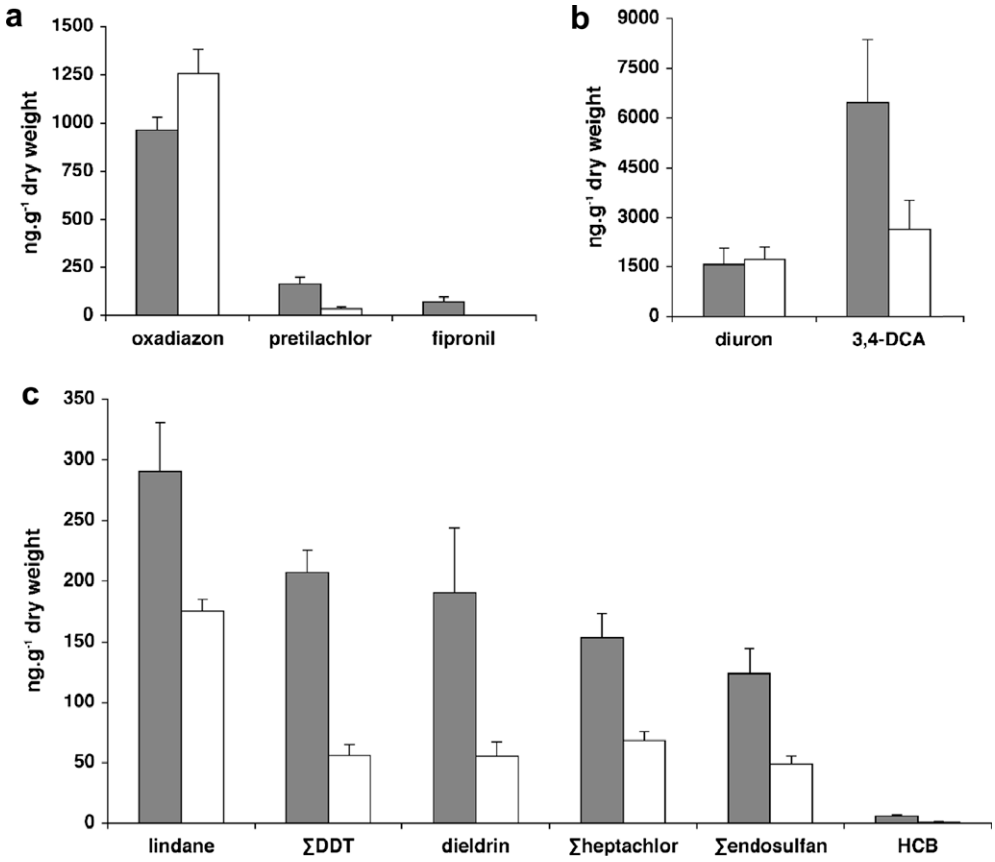

Fig. 2. Bioaccumulation levels, in *Corbicula* caged ■ upstream and □ downstream rice fields in the Rhone delta, of (a) pesticides used currently or which were used during the four last years in rice growing; (b) pesticides used in the Rhone Valley; (c) persistent and banned organochlorine pesticides (OCPs).

Table 2
PCB bioaccumulation in *Corbicula* caged upstream and downstream from conventional rice fields in the Rhone delta. Mean of 18 individuals ± standard deviation; *P*-value from inter-site comparison with the Bonferoni–Dunnett test.

	Upstream	Dowstream	*P*-Value
CB8	235 ± 100	42.6 ± 17.9	0 .09
CB18	6.6 ± 3.2	16.1 ± 13.2	NS
CB28	68.9 ± 21.7	55.7 ± 29.2	NS
CB31	18.4 ± 8.8	nd	0 .07
CB44	68.6 ± 9.0	55.2 ± 15.9	NS
CB52	186 ± 27	166 ± 36	NS
CB70	60.5 ± 20.9	73.2 ± 23.4	NS
CB77	13.4 ± 6.5	8.4 ± 4.7	NS
CB81	10.0 ± 7.2	23.1 ± 11.7	NS
CB101	122.5 ± 9.3	49.5 ± 6.0	<0.0001
CB105	51.5 ± 29.7	14.1 ± 6.3	NS
CB114	18.5 ± 6.8	nd	0.02
CB118	164.7 ± 9.4	56.4 ± 3.1	<0.0001
CB123	87.9 ± 5.2	37.9 ± 2.1	<0.0001
CB126	19.5 ± 9.9	5.3 ± 1.9	NS
CB128	0.9 ± 0.6	4.8 ± 2.2	NS
CB138/137	132.2 ± 8.6	43.6 ± 4.1	<0.0001
CB151	52.7 ± 3.8	20.1 ± 2.2	<0.0001
CB153	482 ± 30	177 ± 8	<0.0001
CB156	14.6 ± 3.9	6.4 ± 1.2	0.08
CB157	10.5 ± 1.2	8.7 ± 0.9	NS
CB167	0.5 ± 0.4	0.5 ± 0.3	NS
CB169	8.6 ± 2.6	4.6 ± 0.6	NS
CB170	0.2 ± 0.2	0.8 ± 0.4	NS
CB180	72.0 ± 5.6	23.8 ± 1.6	<0.0001
CB189	1.1 ± 0.5	0.6 ± 0.3	NS
CB194	2.1 ± 0.7	0.4 ± 0.2	0.03
CB195	4.5 ± 2.0	4.6 ± 1.7	NS
∑PCB	1913 ± 185	898 ± 60	<0.0001
∑Dioxin like	454 ± 42	163 ± 8.9	<0.0001
∑Indicator	1393 ± 147	559 ± 45	<0.0001

3.5. Enzymatic biomarkers

Three enzymatic biomarkers were measured. Fig. 4 shows inter-site variations of enzymatic activities in caged *Corbicula*. The activity of GST, a phase II biotransformation conjugation enzyme, was higher in downstream *Corbicula* ($p < 0.001$) (137 ± 32 μM mg protein^{-1} and 91 ± 21 μM mg protein^{-1} downstream and upstream respectively). PChE neuronal activity, known to be particularly sensitive to organophosphorine pesticides and to carbamates, was higher in irrigation channel than drainage channel *Corbicula* ($p = 0.017$) (35.9 ± 7.03 and 50.5 ± 22.8 μM mg protein^{-1} downstream and upstream respectively). Catalase activity, implied in oxygen free radical metabolism, was equivalent in *Corbicula* from the two sites (304 ± 51 μM mg protein^{-1} vs 298 ± 69 μM mg protein^{-1}).

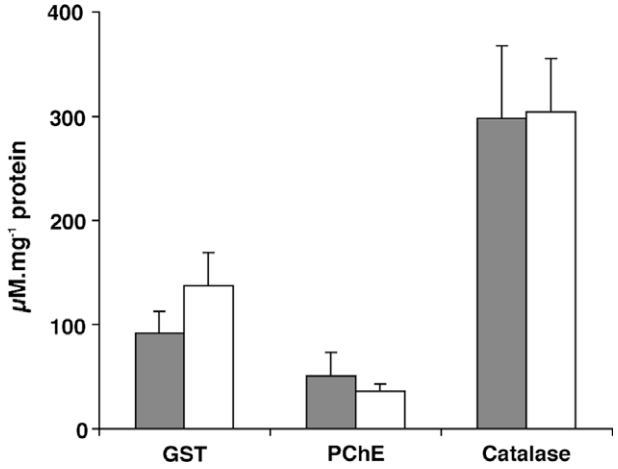

Fig. 4. Activity of three enzymatic biomarkers: glutathione *S*-transférase (GST), propionylcholinesterase (PChE) and catalase, in *Corbicula* from ■ upstream and □ downstream rice fields in Camargue.

4. Discussion

Previously, we described the occurrence of a bioconcentration process of organochlorine pollutants in the trophic web of the Vaccarès Lagoon (NRC) especially due to the agricultural wastewater inflow in this pond. Chemical inputs come from both drainage ditches and from irrigation systems collecting water from the Rhone River. We showed that persistent pollutants, like lindane and endosulfan and PCBs, are biomagnified along the food chain and seriously contaminate top predators like European eels (Oliveira Ribeiro et al., 2005, 2008; Vollaire, 2007). Consequently we developed a biomonitoring programme in the channels flowing in the NRC.

With *Corbicula* as biomonitors, the bioavailability of contaminants in the environment can be highlighted (Narbonne et al., 1999). In the event of multiple contaminations, the biomarker approach provides an integrative answer to assess the chemical risk (van der Oost et al., 2003; Binelli et al., 2006). Biotransformation enzymes, cholinesterase and antioxidant enzymes are valuable

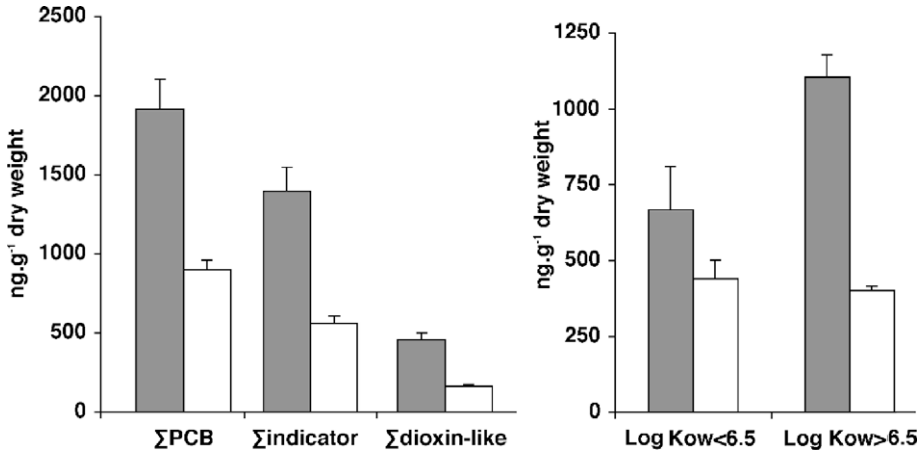

Fig. 3. PCBs bioaccumulation (a) sum of PCBs measured, of indicator and of dioxin-like PCBs and (b) as a function of Log K_{ow}, in *Corbicula* from ■ upstream and □ downstream rice fields in Camargue.

tools (easy to use, cost effective and environmentally valid biological responses). Previously, we showed that catalase activity depended on PCB accumulation and answered by a hyperactivity (Buet et al., 2006). Here, catalase did not exhibit significant inter-site variation unlike GST and PChE. The higher activity of GST, an enzyme participating in the conjugation phase of the biotransformation process, in *Corbicula* caged in downstream rice fields did not reflect impregnation variations, but rather indicated the presence of not investigated contaminants. Concomitantly, the PChE inhibition revealed the occurrence of organophosphorine or carbamate pesticides (Cooper and Bidwell, 2006) in the drainage channel. Such results show that the screening of pollutant chemicals was largely incomplete, excluding cholinesterase inhibitors notably, and requires further analysis at the continuation of these initial investigations. In addition, the occurrence of numerous agents inducing biotransformation processes was not taken into consideration although their presence was signified upstream and downstream.

However, biomonitoring of rice field irrigation and drainage channels showed significant contamination with persistent and banned OCPs in *Corbicula*. Ranked by descending concentrations, we found γ-HCH > DDT > dieldrin > heptachlor > endosulfan. All these compounds are known as very persistent and very biomagnifiable (vPvB) pollutants. Their higher concentration in upstream *Corbicula* points to water transfer via the Rhone River. Their lower concentration in downstream *Corbicula* suggests a removal process between these two sites, for DDT and dieldrin, notably. In France, the use of lindane has been prohibited since July 1998, and before it was mainly used in rice growing. The strong contents of γ-HCH (85.8% of ∑HCH) most likely also reflect recent use in the Rhone valley, which would explain why it occurs in such levels in *Corbicula*. Little work has been published about the use of plants for OCP removal. Macrophytes are generally used as biomonitors (Lytle and Lytle, 2001). Miglioranza et al. (2004), studying the ability of a bulrush (*Schoenoplectus californicus*) to 'sequestrate' OCPs from upstream agricultural fields released in an Argentine Lake, showed that the stems preferentially accumulated the more hydrophilic OCPs such as endosulfan sulphate and HCHs due to their presence in the water column, whereas roots accumulated the more hydrophobic pesticides, such as DDT, concentrated in the sediment. The authors conclude that macrophytes can be used as a tool for OCP remediation.

There has been a bit more work on the potential of plants to accumulate and to degrade DDT (Thomas et al., 2008). Yao et al. (2007) estimated the bioavailability and bioaccumulation of DDE and DDD in rice depend on farming practices, such as flooding of paddy fields, for example. On another hand, Chu et al. (2006) demonstrated that the rice, *Oryza sativa*, accumulated DDT by both passive and active absorption but was unable to completely degrade or transform DDT. Recently, Rose et al. (2008) considered that, in vegetated ponds, aquatic plants contributed to pesticide removal, by enhancing the sedimentation. The dissipation pathways of organic contaminants also included mass transfer to biofilms, adsorption on soil particles, photolysis, hydrolysis and volatilization. In rice fields, alternate wetting and drying cycles facilitate photolysis and volatilization of organic compounds.

The hydrophobic pollutants were transported by a river as a dissolved form or bound to particulate matter. The re-suspension of fine particles of sediment during a flooding can give a dissolved fraction. But the adsorbed fraction in sediments, in SPM or in organisms can be considered as the predominant form (Hilscherova et al., 2007). Although the pollutant partitioning exists under the two forms (dissolved or sorbed) In the Rhône River, (Babut and Miege, 2007) estimated than organochlorines are rather in a sorbed form. In the Rhône Delta, the part of contaminants dissolved or sorbed in suspended matter in the Camargue channels is not currently determined. However, in accordance to (Carvalho et al., 2008), the bioconcentration of organochlorines in the deposit-feeding bivalve molluscs could be mainly due to the ingestion of sediment particles followed by digestion and absorption of sediment associated organic matter.

Oxadiazon and pretilachlor are chemicals used to control weeds in flooded rice in Camargue and fipronil was used to control chironomids before 2004 (Mesleard et al., 2005). At the present time, its use in crop protection is under restrictions of employment, although this is currently being discussed in the framework of European regulation. Recently, Comoretto et al. (2007) showed that oxadiazon and pretilachlore were among the major pesticides in drainage water at the mouth of the Fumemorte Channel and that their concentrations were similar, i.e. 0.26 and 0.31 μg L^{-1}, respectively. Conversely, the data from the present study revealed a difference in the bioaccumulation of the two chemicals in bivalves. Indeed, oxadiazon bioaccumulation was 25 times greater than pretilachlore concentration. Additionally, the bioconcentration process of pesticides currently in use in the rice plantations is difficult to assess. Nevertheless a reduction of pretilachlor (and to a lesser extent fipronil) was observed in downstream *Corbicula* compared to upstream bivalves.

The documented extraction of PCB from soil by plants exhibits random effectiveness (Macek et al., 2000; Singer et al., 2003; Chekol et al., 2004; Mackova et al., 2006; Dercová et al., 2007; Smith et al., 2007). Among these authors, Zeeb et al. (2006) explained that *Curcubitacea* are effective plants from wetlands but an association of several varieties would offer a better phytoextraction potential. In any case, bioremediation constitutes an alternative method for purification of PCB-contaminated ecosystems. Moreover, following (Luo et al., 2008) which showed that in terrestrial soil, microorganisms are able to remove more PCBs than in river sediment, we also speculate about the ability of rice fields to promote growth of efficient microorganisms.

In the present paper, we demonstrated that PCB bioaccumulation was generally lower in downstream *Corbicula*. In addition, the more concentrated PCBs were highly chlorinated congeners, and they showed greater decrease (>50%), whatever their toxic power (PCB indicators and dioxin-like). Furthermore, it is known that highly chlorinated PCBs are more difficult to degrade than less chlorinated PCBs (Rezek et al., 2007). In the process of clearing organic chemicals from the environment, plants employ various mechanisms including degradation, adsorption, volatilization and accumulation. Several studies have shown a sequestration of PCBs in various parts of plants. For example, Chu et al. (2006) estimated that PCBs may accumulate selectively in different organs showing a translocation process in common reed and rice. Such data contribute to renewed interest and feed hopeful speculation about natural removal of PCBs.

5. Conclusion

A biomonitoring assay was initially performed to clarify the responsibility of rice plantations in the contamination of trophic guilds in the Vaccarès Lagoon located in the centre of a deltaic zone which presents both an incomparable biodiversity and an exceptional agricultural activity. Bivalves caged in channels upstream and downstream from a conventional rice growing area showed that chemicals used in rice growing bioaccumulated and that several organochlorine compounds potentially brought by the Rhone River showed an impregnation decrease in *Corbicula* caged downstream. These observations reveal the ambivalent role of the rice plantation in pollution of adjacent ponds. In this context, the immersed plants in wetlands seem to show an exploitable power (Williams, 2002). Nevertheless, in order to ascertain the real impact of rice growing in a speculative phytoremediation process of

Rhone River water before discharge into the Camargue Lagoons, we target to explain the mechanisms by which chemicals in bioindicator species disappear and to analyze the role of rice, its tissues, and the diverse compartments of the rice plantation as well as the influence of cultural practices in a potential removal process. Previously, we have showed that the Vaccarès Lagoon, the largest waterbody in NRC, was contaminated by persistent pollutants from multiple sources (Roche et al., 2000, 2002a,b; Oliveira Ribeiro et al., 2005, 2008). A part of these compounds is brought by irrigation channels of rice fields. If sustained in the future, this pollution could really endanger the health of the whole wetland ecosystems and menace the populations of fishes and of piscivorous birds, located at the highest level of the biomagnification process for xenobiotics, and major component of its protected biodiversity.

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