DETOXIFICATION EXPERIMENT IN NATURAL CONDITIONS FOR MANILA CLAM (*Tapes philippinarum*) CONTAMINATED BY POPS IN THE LAGOON OF VENICE

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Introduction

Since decades the Venice Lagoon has been receiving discharges of many classes of pollutants from its drainage basin and from urban and industrial areas laying on lagoon borders. In particular, in the last century Porto Marghera (Figure 1) was developed into one of the largest industrial zones in Europe and mainly from this site, dioxins and dioxin-like compounds, as well as other classes of pollutants, were released into the environment. POPs have therefore been accumulating in lagoon sediments and concentrations higher than 2500 ngI-TE $kgdw^{-1}$ were found in sediments of the Industrial Zone's channels. Recently this pollution became subject of concerns for the possible threat to human health due to the direct uptake of POPs through consumption of harvested fish and shellfish species[1]. In particular, the invasive species Manila clam (*Tapes philippinarum*) is the target of an intensive fishing activity and the Venice Lagoon alone provides to the fish market around 40000 metric tonnes of Manila clam per year, which is more than 50% of the Italian clam production. *Tapes philippinarum* was introduced in the Venice Lagoon in 1983 for enhancing shellfish production and spread in the whole lagoon, including the area around the Industrial Zone, which is now an important area for recruitment and growth of this species. The harvesting of *Tapes philippinarum* is not allowed nearby the Industrial Zone, but specimen illegally caught in this highly polluted area might reach consumers. In order both to discourage the illegal fishing and maintain exploitation opportunities for this resource, detoxification experiments were prompted by the local Administrative Council, i.e. the Regione Veneto. These experiments aim at assessing the effectiveness of a culture-based fisheries strategy based on the removal of *Tapes philippinarum* specimen from the Industrial Zone and their resettlement in cleaner areas of the lagoon. A previous experiment, conducted in summer 2004, showed a very rapid decline in the toxicity of clam flesh when specimen are reared in sediments with low POPs contamination[2]. Here, we present data regarding a new experiment conducted with the support of the Provincia di Venezia in the winter 2006, when the lower temperature of lagoon's waters hampers the detoxification and the decline of toxicity is slower. This last experiment was conducted in two sites of the lagoon and the sampling frequency was higher than the 2004 experiment, thus allowing a more robust evaluation of the detoxification processes.

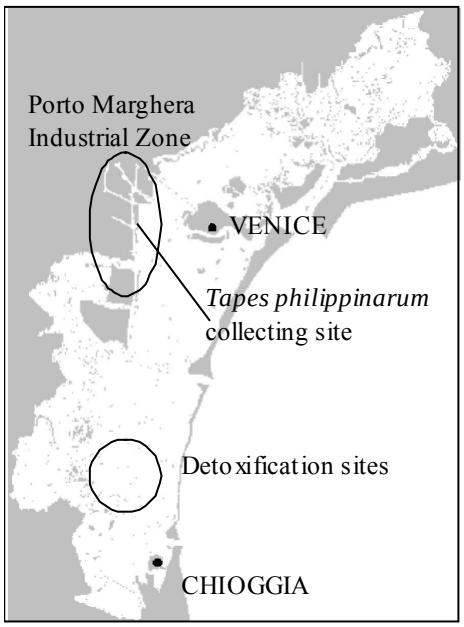

Figure 1. Map of the Venice Lagoon reporting the sites where *Tapes philippinarum* were collected (in the Industrial Zone of Porto Marghera) and reared (southern lagoon) for the detoxification experiment.

Materials and Methods

Contaminated specimen of *Tapes philippinarum* at minimum marketable size (25mm of length) were collected in the area surrounding the Industrial Zone and reared in two areas of the southern part of Venice Lagoon (Figure 1), which are reserved areas for rearing clams and are characterized by sediments with low contamination by industrial sources. Clam specimens were kept in 50cm x 100cm plastic nets, with a mesh size of 5mm, which were laid on the sediment. A biomass of about 50 kgww of clams was used in the experiment, keeping the density in each net approximately 1000g ww m^{-2}. Detoxification experiment last for 60 days: every 5 days (10 days toward the end of the experiment)

specimens were collected and concentrations of PCDD/F, PCB and HCB were determined in clam flesh. Therefore concentrations, expressed on lipid weight basis, were determined at day 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50 and 60.
Moreover, superficial sediments (0-10 cm) were sampled in the site where clams are collected (Industrial Zone) and in the two sites chosen for detoxification (Cooperative1 and Cooperative 2) and concentrations of PCDD/F, PCB and HCB were measured on a dry weight basis. The sediment samples were transported on ice and stored at 4°C. They were thoroughly mixed with a stainless steel spatula, in order to obtain 10.0±0.1 g sub-samples. The moisture content of each sediment sample was determined by drying a separate sub-sample of sediment overnight in a conventional oven at 105°C. The clam flesh was rinsed with distilled water, homogenised, freeze-dried, grinded, and sub-samples of 10.0±0.1 g were transferred to glass beker. Each sediment and clam sub-sample was first spiked with a series of 15 $^{13}C_{12}$-labeled 2,3,7,8 PCDD/F (EDF8999) with a series of 12 $^{13}C_{12}$-labeled PCB (EC4937), with $^{13}C_{12}$-HCB (CLM351) substituted isomers, as internal standards. The quantitative determination of PCDDs/PCDFs and PCB-HCB was performed by an isotope dilution method using relative response factors previously obtained from five standard solutions injections, as recommended by the US-EPA [3,4]. The reader is referred to the previous detoxification experiment for a more detailed description of the analytical methods used [2].

Results and Discussion

	Ind. Zone	Coop. 1	Coop. 2
PCDD/F WHO-TE (ng/kg dw)	37.063	0.240	0.437
PCB WHO-TE (ng/kg dw)	2.990	0.006	0.021
PCDD/F+PCB WHO-TE (ng/kg dw)	40.052	0.245	0.458
OCDF/OCDD	4.692	0.395	0.304
PCB Total (like aroclor) (µg/kg dw)	43.30	0.25	0.70
HCB (µg/kg dw)	7.28	0.05	0.06

Table 1.
Summary of concentrations of POPs for sediments sampled in the site where clams were collected (Industrial Zone) and in the two sites where clams were reared during the detoxification experiment (Cooperative 1 and 2).

Table 1 reports a brief summary of POPs concentration measured in surface sediment samples. POPs concentrations in the sediments from the Industrial area showed very high contaminations with PCDD/F+PCB toxicity of 37.063+2.990 ngWHO-TE/kg dw. In contrast, the PCDD/F+PCB concentrations in the sediments from the southern Lagoon samples showed toxicity two order of magnitude lower, i.e. 0.240+0.006 and 0.437+0.021 ngWHO-TE/kg dw respectively for surface sediments of Cooperative 1 and Cooperative 2. Moreover, HCB and Aroclor showed concentrations two orders of magnitude higher in surface sediments from Industrial Zone than in those from southern lagoon (Table 1).

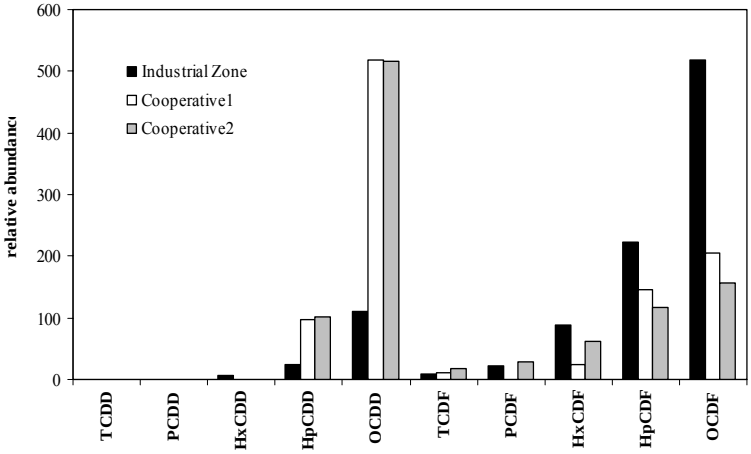

Figure 2.
Relative abundance of dioxin congeners for superficial sediments where *Tapes philippinarum* specimens were collected (Industrial Zone) and where clams were reared (detoxification sites). Dioxin fingerprints clearly show contamination from chlorinated productions for the Industrial Zone sediments.

Interestingly the ratio between dioxins congeners (OCDF/OCDD) was much higher in highly contaminated sediments (4.692) than in the southern lagoon ones (0.395-0.304). This evidences the difference source of contamination for sediments from the industrial and the southern area of the lagoon that can be demonstrated by

looking at the fingerprints of the dioxins congeners, reported in Figure 2. Sediments from industrial area are highly influenced by chloro-alkali, PVC and chlorinated hydrocarbons productions while sediments from the southern lagoon are contaminated by urban discharges from the city of Chioggia, by atmospheric depositions, and by leakage of hydrocarbons and incomplete fuel combustion from boats [1,5,6].
Differences between POPs' concentrations in the sediments from the two detoxification sites were within the measurement errors. Moreover, concentrations of POPs during time for clams reared in the two detoxification sites were similar, therefore, in the following, data presented will regard the mean of concentrations measured for POPs in clam flesh in the two detoxification sites.

sample	time	WHO-TE PCDD/F	WHO-TE PCDD/F	WHO-TE PCB	WHO-TE PCB
	(days)	(pg/g ww)	(pg/g lipid)	(pg/g ww)	(pg/g lipid)
T0	0	3.361	617.173	0.297	54.466
T1	5	1.212	146.094	0.190	22.864
T2	10	0.707	176.440	0.103	25.780
T3	15	0.451	74.504	0.065	10.790
T4	20	0.203	26.616	0.056	7.344
T5	25	0.244	37.452	0.079	12.069
T6	30	0.161	26.535	0.068	11.157
T7	35	0.163	20.876	0.067	8.605
T8	40	0.129	16.752	0.072	9.305
T9	45	0.136	18.341	0.058	7.896
T10	50	0.110	14.232	0.056	7.274
T11	60	0.091	11.904	0.057	7.377

Table 2. Concentrations of dioxins and PCBs in clam flesh during the detoxification experiment. Concentrations are expressed in toxicity equivalents (WHO-TE) on wet weight and lipid basis. Initial samples (T0) represents clams collected in the Industrial Zone before the start of the detoxification experiment.

Average concentration in flesh of clams collected in the Industrial Zone was 617.17 and 54.47 pgWHO-TE/g lipid respectively for PCDD/F and PCB. The results of the detoxification experiment are summarized in Table 2, which shows the toxicity of PCDD/F and PCB in clam flesh during time, expressed on both wet weight and lipid weight basis. Previous detoxification experiment conducted in summer 2004 showed that equivalent toxicity decreasing of an order of magnitude in ten days and that no substantial decrease was observed after 30 days of detoxification [2]. Present data reported in Table 2 clearly suggest that during winter the detoxification process takes much longer time: dioxins toxicity decrease of an order of magnitude after 15-20 days of detoxification while abatement of PCB toxicity appears to be still significantly active 30 days after detoxification started.
In order to compare this detoxification experiment with the previous one done in summer 2004, biological half-lives and decay rates of PCDD/F and PCB were estimated using as background value for toxicity (y_b) the average of the last five measurements and estimating the detoxification rate from the first sampling (T1) [2]. Therefore, decay rate, k, was estimated using the expression, $k= \ln[(y_{T0}-y_b)/(y_{T1}-y_b)]/(t_1-t_0)$, in which y_{T0} and y_{T1} represent, respectively the equivalent toxicities at time t_0 and t_1. Biological half-life (ε) is then estimated from decay rate as $\varepsilon= [-\ln(1/2)]/k$: both estimates for toxicity of PCDD/F and PCB are reported in Table 3.

WHO-TE	PCDD/F	PCDD/F	PCB	PCB
	on ww basis	on lipid basis	on ww basis	on lipid basis
2006 detoxification experiment (winter)				
y_b (pg/g)	0.126	16.421	0.062	8.091
k (day^{-1})	0.218	0.307	0.122	0.229
ε (day)	**3.174**	**2.261**	**5.690**	**3.030**
2004 detoxification experiment (summer)				
k (day^{-1})	0.325	0.337	0.277	0.302
ε (day)	**2.135**	**2.055**	**2.505**	**2.299**

Table 3.
Estimation of half-life and decay rate for PCDD/F and PCB toxicity in the detoxification experiment conducted in winter 2006 and comparison with previous estimates for summer 2004 [2].

Half-lives are, on lipid basis, 2.26 day^{-1} and 3.03 day^{-1} respectively for PCDD/Fs and PCBs, thus slightly greater than the 2.06 day^{-1} and 2.30 day^{-1} estimated in the detoxification experiment done in 2004 [2]. Moreover, half-lives in wet weight basis were substantially greater in winter experiment (2006) than in the summer one (2004), being 3.17 day^{-1} and 5.69 day^{-1} for PCDD/F and PCB respectively. These results show that temperature is important factor controlling the metabolic activities of *Tapes philippinarum* and thus is greatly influencing detoxification process that are, therefore, much slower in wintertime.

The relative abundances of dioxin congeners in the flesh of *Tapes philippinarum* represent the fingerprint of contamination source. Dioxin fingerprints for clam flesh during detoxification are reported in Figure 3, which shows, in particular, a decrease of relative abundance of OCDF and an increase of relative abundance of OCDD during detoxification (thus the ratio OCDF/OCDD is decreasing over time). According with dioxin fingerprints for surface sediments previously shown and with the strong relationship between clam and sediment contamination found in other studies [7], this result clearly shows that the detoxification interests the industrial contamination.

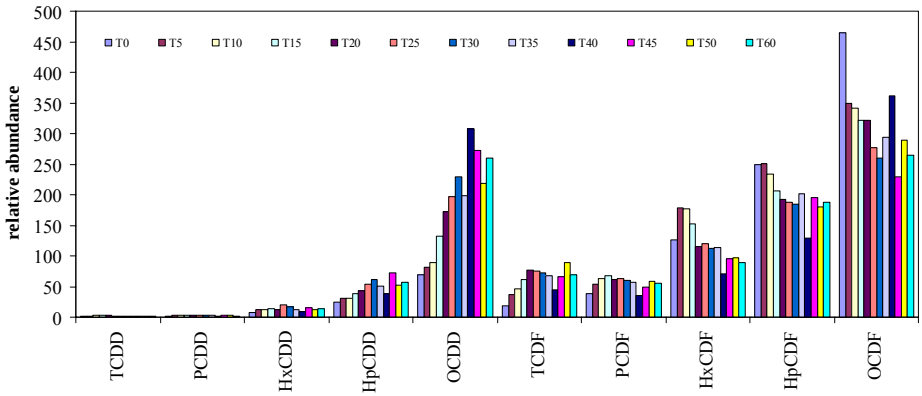

Figure 3.
Relative abundance of dioxin congeners in the clam flesh over time represent the change in the contamination fingerprint during detoxification process.

Conclusions

PCDD/Fs and PCBs in flesh of *Tapes philippinarum* contaminated by industrial pollution and reared in low contaminated sediment underwent a slower decline than previously found [2], due to the reduced metabolic rates in winter, and therefore, detoxification rates and biological half-lives of PCDD/Fs and PCBs found in this experiment should be considered more precautionary. Findings presented show the potential of natural detoxification, and could represent a solution for the illegal fishing carried out in the Industrial Zone, which might be used as nursery area for *Tapes philippinarum*, to be reared in cleaner sediments of the southern lagoon and then consumed safely.

Acknowledgments

We thank Provincia di Venezia (Settore Caccia Pesca e Polizia Provinciale), for supporting this experimental activity and for permitting the use of data presented here. The Provincia di Venezia is the solely owner of data presented and allowed their use for scientific purposes only.

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