

## Correlation between POPs in sediment and edible bivalve in the Lagoon of Venice and estimation of the daily intake

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### Introduction

The lagoon of Venice, which is located in the Northern Adriatic Sea, is the largest Italian Lagoon, covering an area of about 550 km<sup>2</sup>. Its average depth is about 0.7 m, but its morphology is characterized by the presence of large shallow areas and by a network of deeper channels. As can be seen in Fig. 1, three narrow inlets connect the lagoon to the Adriatic Sea. The lagoon is conventionally partitioned into three sub-basins, which are separated by two watershed, along which the tidal velocity are low. In the last century the area of Porto Marghera, opposite the city of Venice, was converted into one of largest industrial area in Europe. As a result of the industrial activities, dioxin and dioxin-like compounds, as well as other classes of pollutants, were released into the environment. The lagoon sediment represented the main sink for this contaminants: in fact, concentrations higher than 2500 ng(I-TE)/kg were found in the sediment in the channels of the industrial area. At present, *Tapes philippinarum* is the target of an intensive fishing activity, which severely damages the lagoon sediment and the benthic community. Even though fishing is not allowed nearby the Industrial area, illegal catches of contaminated clams are still being commercialized. As a consequence, the risk for health of regular consumer of this bivalve should be estimated. In order to assess the risk and to reassure the consumers, In the year 2002, the main local fishermen association, CoVeAILa, has set up a monitoring program of PCDD/F, PCB dioxin-like and HCB both in the sediment and in the flesh of the edible bivalve *Tapes philippinarum*. In this paper, we present and discuss the results which were obtained in the year 2003.

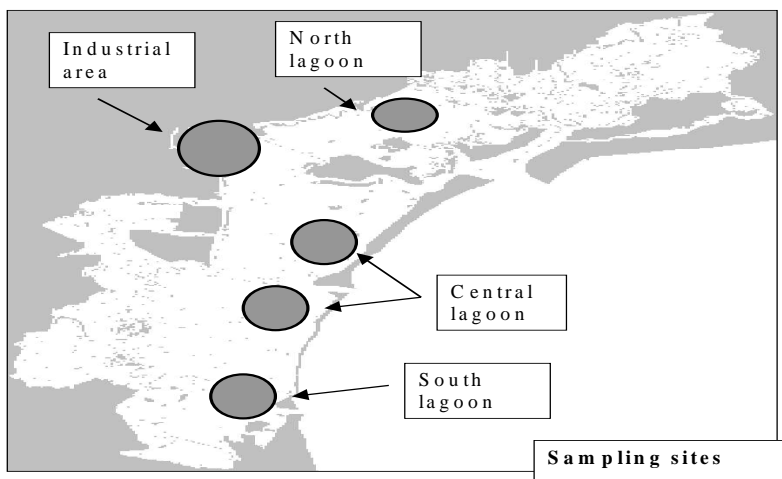


Fig. 1. Sediment and clam sampling sites

## Methods

The samples of sediment and *Tapes phillipinarum* were collected in the North, Central and South lagoon within the three areas shown in Fig. 1. The exploitation of *Tapes phillipinarum* in these areas is presently carried out in a culture based fishery regime. Each sediment sample reflects, in fact, the composition of ten sub-samples of the surficial sediment, first 10 cm, which were separately taken and then homogenized. Seven sediment and clam samples were collected in the Northern area and in the Central one, whereas eighteen samples were taken in the Southern area. The location of the sample sites were determined using a GPS system. Clam samples concerned only specimen larger than the market size, which is 25 mm and is usually reached by 18 months old specimen. The concentrations of PCDD/F; PCBs; HCB in all sediment samples were calculated on a dry weight basis. 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 concentrations in the clam flesh are expressed on a wet weight basis.

All glassware was washed with basic detergent, rinsed with distilled water, treated with a solution of ammonium persulphate 350 g/L in sulphuric acid (98%) and rinsed twice with distilled water and acetone. Subsequently, the cleaned glassware was treated with dimethyldichlorosilane 5% in toluene, rinsed twice with distilled water and acetone, heated to 300°C for 3h, and covered with aluminium foil. 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  $10.0 \pm 0.1$  g sub-samples. The clam flesh was rinsed with distilled water, homogenised, freeze-dried, grinded and  $10.0 \pm 0.1$  g sub-samples were transferred to glass beaker. Each sediment and clam sub-sample ( $10.0 \pm 0.1$ g) was first spiked with a series of 15  $^{13}\text{C}_{12}$ -labeled 2,3,7,8 PCDD/F (EDF8999) with a series of 12  $^{13}\text{C}_{12}$ -labeled PCB (EC4937), with  $^{13}\text{C}_{12}$ -HCB (CLM351) substituted isomers, as internal standards, mixed with Spe-ed Matrix. The extraction by ASE 200 (DIONEX Sunnyvale, CA) was performed with 50 mL of toluen at 135 °C, 2000 psi, 7 min. heat-up and 2 cycles of 10 min static time. The extracts were transferred to hexane before the clean up treatment. The extracts were transferred to hexane and then were treated with sulphuric acid (98%) and potassium hydroxide (20%) in a 100

mL separatory funnel The extracts were firstly spiked with  $^{37}\text{C}_4$ -labeled 2,3,7,8 PCDD (EDF6999) and with  $3\text{ }^{13}\text{C}_{12}$ -labeled PCB (EC4978) and then cleaned up using the automatic system, Dioxin Prep (Fluid Management System Inc) with pre-packed disposable columns containing multilayer silica, alumina and carbon for PCDD/F, PCB and HCB. The HRGC/HRMS analyses were conducted using a HP 6890 plus gas chromatograph coupled to a Micromass Autospec Ultima mass spectrometer operating in EI mode at 35 eV and with a resolution of 10.000 (5% valley). PCDD/F Sample injections were performed in the splitless mode on a 60 m DB5 ms column (J&W 0.25 mm ID, 0.25  $\mu\text{m}$  film) and on a 60 m Rtx 200 (Restek 0.25 mm ID, 0.25  $\mu\text{m}$  film) for verification. The quantitative determination of PCDDs/PCDFs was performed by an isotope dilution method using relative response factors previously obtained from five standard solutions injections (EDF 9999 Cambridge Isotope Laboratories, Woburn, MA.), as recommended by the US-EPA<sup>(1)</sup>. The quantitative determination of PCB-HCB was performed by an isotope dilution method using relative response factors previously obtained from five standard solutions injections (EC1668 Cambridge Isotope Laboratories, Woburn, MA.), as recommended by the US-EPA<sup>(2)</sup>. The data concerning the Industrial area were instead collected in the framework of a monitoring plan which was carried out by public authorities for assessing the contamination levels of sediment and clams. These data were previously presented and discussed in <sup>(3,4)</sup>.

## Results and discussion

The ranges and the average values of the toxicity equivalent in the clam flesh in the three monitoring area are compared with those concerning the clam sampled nearby the Industrial area in Fig. 2. As one can see, the contamination levels in the three fishing areas are quite similar and approximately an order of magnitude lower than those found in the canals of the industrial zone, where the fishing is forbidden. The relative concentrations of the main congeners, expressed as percentages, in both sediment and clams, i.e. the fingerprints, are shown in Fig. 3a-3d. The visual inspection of the finger-prints strongly suggest that the sources of contamination in the three monitored areas are different. In fact, the finger-print concerning both the sediment and the clams nearby the Industrial area, Fig. 3a, was characterized by the highest percentage of OCDF, about 70% in the sediment, which was and still is released in the environment as a result of the chloro-alkali, PVC and chlorinated hydrocarbons productions <sup>(3)</sup>. The percentage of OCDF was lower in the other three areas and reached its minimum in the South Lagoon, where the finger-print was characterized by the highest percentage of OCDD. This compound, in fact, is usually found in the discharges of the urban waste water treatment plants, and can be released in the environment also by combustion processes and boats engine. The other two finger-prints here presented were characterized by similar percentages of the above congeners. This finding indicates that the central and north lagoon were affected by both urban waste water and industrial pollution. However, the fact that the percentages concerning OCDF are higher than the OCDD ones suggest that in both areas the contamination related, directly or indirectly, to industrial activities<sup>(3,4)</sup>. It is also interesting to note that the finger-prints in clam and sediment pertaining to the same area were remarkably similar, and characterized by concentrations of TCDF higher in the clam than in the sediment. This finding supports the hypothesis that the sediment was still the main source of clam contamination and suggested the presence of a quantitative relationships between the equivalent toxicities in the abiotic and biotic compartment. In fact, as one can see in Fig. 4a and 4b and in Table 1, the regression between the logarithms of the WHO-TE in clam flesh and sediment was found to be statistically significative for both PCDD/F and PCB.

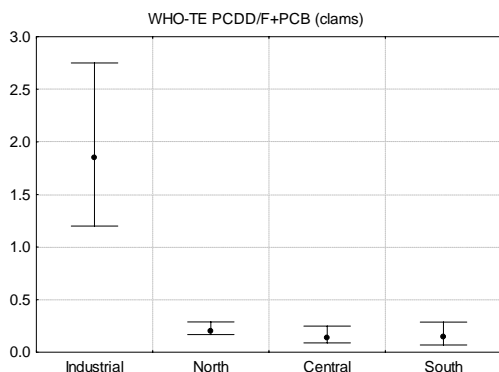


Fig. 2. Range and average pgWHO-TE/g w.w. (PCDD/F+PCB) in the clam flesh in the three sampling areas in the year 2003, in comparison with those concerning the industrial area.

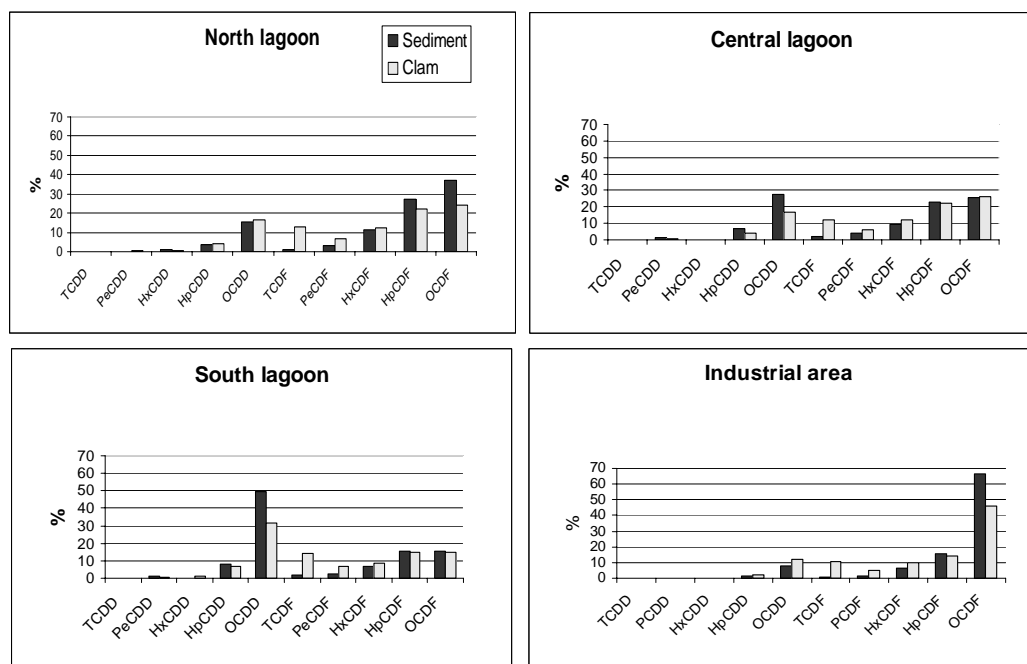


Fig. 3. Finger-prints in the sediment and the clam flesh in the three sampling areas in the year 2003, in comparison with those concerning the industrial area.

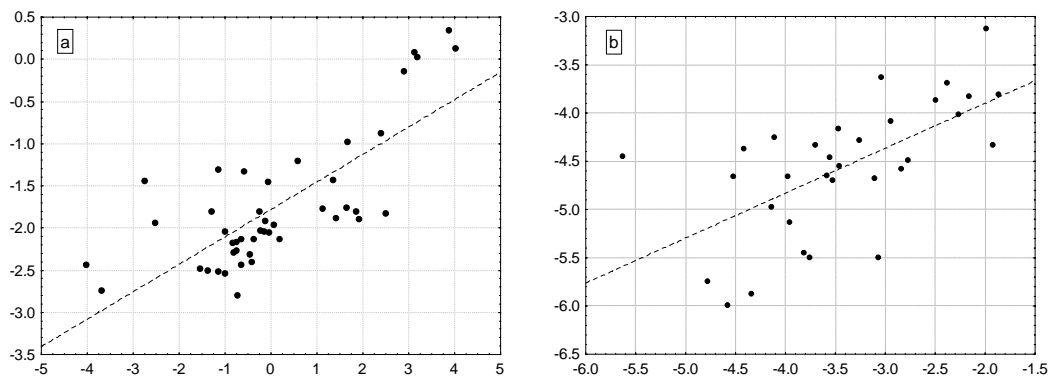


Fig. 4. Regressions between the logarithm of the WHO-TE-PCDD/F, Fig. 4a, and PCB, Fig. 4b, in clam flesh, dependent variable, and sediment, independent variable.

Tab. 1. Results of the regression analysis.

	$\beta_0$	Std.err $\beta_0$	$\beta_1$	Std.err $\beta_1$	$R^2$
PCDD/F	-1.77860	0.077509	0.32538	0.042655	0.57
PCB	-2.96623	0.377246	0.46585	0.106741	0.37

### Conclusion

The results presented in the previous section show that the log-log regression model here proposed explains, respectively, 0.57 and 0.37 of the variances of the equivalent toxicities, due to the presence of dioxins and dioxin-like PCBs in the clam flesh on the basis of the respective equivalent toxicities in the sediment. From the point of view of the monitoring purposes, this means that, if the regression model will be corroborated by further observations, it will be possible to predict the equivalent toxicity on the basis of sediment data with a reasonable degree of accuracy, thus saving money and time.

As far as the contaminations pathways are concerned, the fact that the finger-prints concerning the sediment and the clams which were sampled in the same areas were very similar indicates that the sediment is the main source of contamination. Furthermore, the presence of an higher percentage of OCDF in the central lagoon samples compared with the southern lagoon ones suggests that the sediment of the former area were contaminated by industry-related activities. To this regard, it also interesting to note that the same feature is shared by the fingerprint which characterizes the northern lagoon samples. This finding, in this basin, may indicate the presence of secondary pollution sources, such as unauthorized industrial waste landfills<sup>(3)</sup>.

From the point of view of the health risk for local regular clam consumer, it should be remarked that the equivalent toxicities within the three monitored areas are very similar and not very high. In fact, if one multiply the average, 0.153 pg WHO-TE/g w.w. by a daily dose of 15g/day, one obtains a daily intake of 2.3 pg/day. Therefore, the choice of restricting the fishing activity to those areas was certainly beneficial for the consumers.

However, it must be pointed out that the situation is different if one takes into consideration the fact that the illegal fishing of *Tapes philippinarum* is still practised in the most contaminated area and these catches are still commercialized on the local market. As shown in<sup>(5)</sup>, in this case, the

consume of *Tapes philippinarum* would represent a threat for the health of a small percentage of local regular consumer.

## References

- 1 US EPA Method 1613B/94: Tetra-Through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS.
- 2 US EPA Method 1668A/99: Chlorinated Biphenil Congeners in water, soil, sediment, and tissue by HRGC/HRMS
- 3 S. Guerzoni, S. Raccanelli., “La laguna ferita” pp19-30, pp 41-48, pp55-64, 2003 ed. Cafoscarina, Venezia, Italy.
- 4 Ferrari G., Raccanelli S., 1999-2000. Perizia sulla presenza e la provenienza di diossine nell’ambiente lagunare veneziano. CTU PROCEDIMENTO N. 3340/96 R.n.r. (n). [www.ilpetrolchimico.it](http://www.ilpetrolchimico.it).
- 5 Zapponi A., gennaio 2003, “Pericolosità e rischi delle sostanze chimiche rinvenute in vongole sequestrate a Venezia”, CTU PROCEDIMENTO N. 4542/01 R.n.r. (n), Procura di Venezia.