SPICOSA: Policy Issue - SSA 7.15 Venice Lagoon: Acquiring useful tools to shellfish fishery management in the Venice Lagoon System

ECOTOXICOLOGICAL EVALUATION OF CLAM HARVESTING AREAS

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OBJECTIVE

Since several ecotoxicological studies were performed in the Lagoon (since the 1980s) we will synthesise the main evidences from ecotoxicological indicators in the areas of clam harvesting. Three groups of bioindicators were defined (Fränzle, 2006): a) test organisms, used in toxicity bioassays which involve the deliberate exposure of test animals to contaminants or contaminated materials (Hahn, 2002); in an aquatic environment, the species is generally exposed in controlled conditions to an environmental sample containing mixtures of pollutants at unknown concentrations in order to verify possible toxic effects (Volpi Ghirardini and Pellegrini, 2001); b) accumulation indicators, which must dispose of a fairly high amount of strain resistance enabling them to incorporate for a considerable time, depending on the uptake-excretion ratio, potentially toxic substances without injury (Fränzle, 2006); c) reaction indicators, which respond (relatively) quickly and in an observable or measurable manner to physical or chemical stress; these indicators are generally known as biomarkers, which are biochemical, physiological or behavioural variations measured in a tissue, biological fluid or the whole organism (individual or population) evidencing exposure and/or effect to one or more contaminants (Depledge and Fossi, 1994).

PREFACE

We recently provided the state of the art on the ecotoxicological indicators used to assess the environmental quality of Venice lagoon with regard to chemical pollutant impacts and we synthesized the main evidences deriving from their application (Losso and Volpi Ghirardini 2009, in press). Italian and international scientific journals, reports from research and monitoring programs and Ph.D. theses have been consulted in order to take stock of the bioindicators applied in the Venice lagoon. As a results, we put on evidence that since 1990 many organisms have been employed as ecotoxicological indicators, including bacteria (1 species), algae (9 species), molluscs (8 species), polychaetes (3 species), crustaceans (7 species), echinoderms (2 species) and fishes (9 species). Anyway the most used are those reported below.

About toxicity bioassays, the toxicity test on sediment with the bacterium *Vibrio fischeri* is one of the most applied to the entire Venice lagoon. Another widely-used bioindicator is the echinoid *Paracentrotus lividus* for which data are available with the acute sperm cell toxicity bioassay and the sub-chronic embryotoxicity bioassay at lagoon scale for the elutriate (60 sites) and at small scale (15 sites for sperm cell test, 7 sites for embryo test) for the porewater. The embryotoxicity bioassay with the bivalves *Crassostrea gigas* and *Mytilus galloprovincialis* has been applied in recent years both on elutriates and on porewaters from the same samples where the toxicity bioassays with *P. lividus* had previously been conducted. Another toxicity bioassay recently used for the entire Lagoon was the survival test with the amphipod *Corophium* sp.
In order to integrate available toxicity data for the Venice lagoon, the Weighted Average Toxicity Index (WATI) was recently developed, so that sediment toxicity is classified in five toxicity classes (absent, low, medium, high, very high) and can easily be visualized on a map for decision-makers (Volpi Ghirardini et al. 2007; Losso et al., 2009 submitted). The WATI integrates the results from 5 toxicological core-metrics (*V. fischeri* toxicity bioassay on whole sediment, sperm cell toxicity bioassay with *P. lividus* on porewater, embryo toxicity bioassay with *P. lividus* on elutriate, embryo toxicity bioassay with *C. gigas* on porewater, embryo toxicity bioassay with *M. galloprovincialis* on

elutriate). For this policy issue, we will improve the WATI also with the survival test with the amphipods *Corophium* sp.
About bioaccumulation studies in the Venice lagoon, they were focused mainly on the mussel *M. galloprovincialis,* the clam *T. philippinarum*, the polychaete *Hediste diversicolor* and the fish *Zosterissessor ophiocephalus.* Bioaccumulation data regard both heavy metals and the main classes of organic compounds (PCBs, PAHs, PCDD/F, organochlorinated pesticides) for organisms sampled in many lagoon sites.

About biomarkers, the most often used species at lagoon-scale was the bivalve *M. galloprovincialis,* using both active and passive approaches and applying biochemical, cellular, physiological and genetic damage biomarkers. The studied for the clam *T. philippinarum* are also numerous and on a large-scale, covering both active and passive monitoring; biochemical, physiological and cellular biomarkers were applied, but the genetic damage has not yet been studied. The benthic fish *Z. ophiocephalus* was used both for genetic biomarkers (DNA adducts) and for fish biomarkers, but the spatial extension of the investigated sites is still limited as it depends on species area distribution.

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The Figure 1 evidences the sites for which data on sediment toxicity with toxicity bioassays are available (by peer-reviewed papers) in the period 2002-2006; red lines show the areas for clam harvesting according to the “Plan for the sustainable use of alieutic resources” (Gral, 2008).

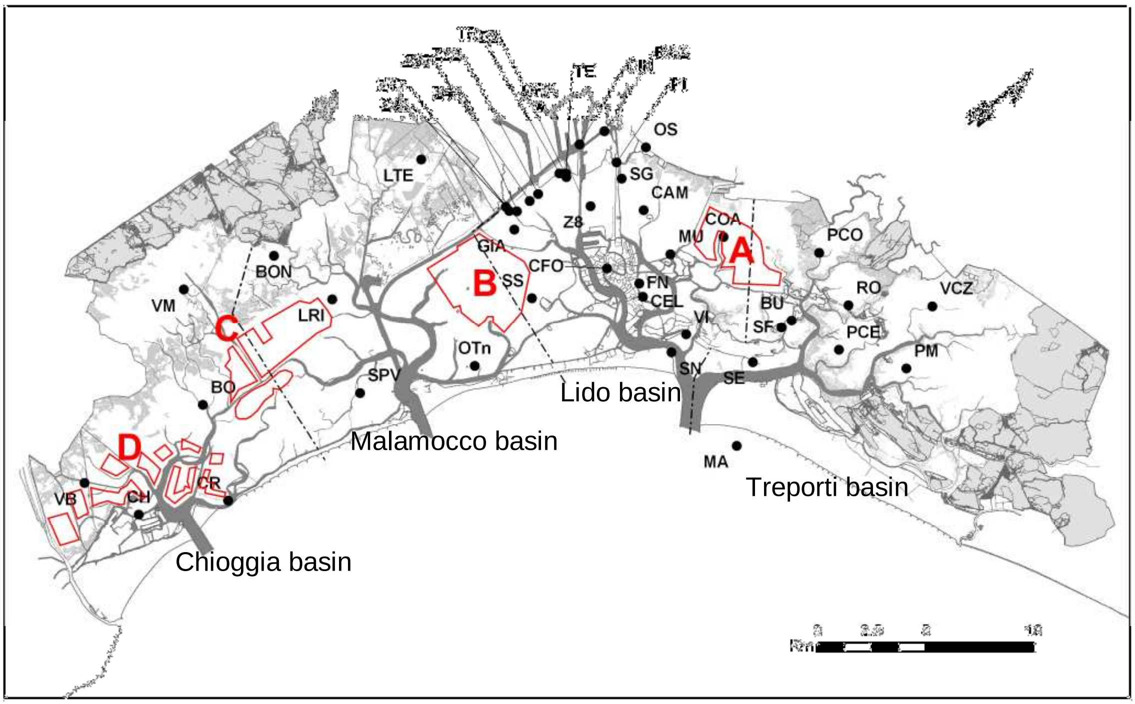


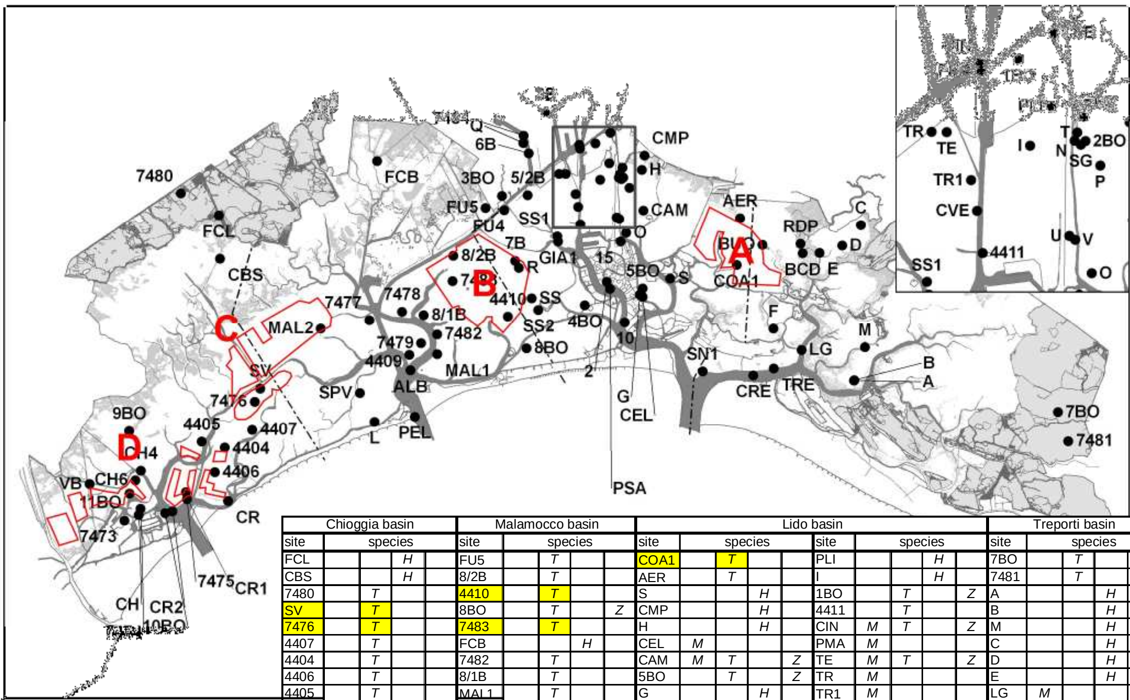
Figure 1. Map of the Venice lagoon indicating the location of the sampling sites for which information with toxicity bioassays are available. Dotted lines indicated the watersheds which individuated the four basins. Red lines showed the areas for clam harvesting.

In Table 1 we synthesized the toxicity classes from each toxicological core-metric and from WATI for these 45 sites, dividing them into the four lagoonal basins (after the site acronym we reported the seasonal sampling, i.e. s= summer, w=winter, and the sampling year). In the Treporti basin, the WATI toxicity classes range from absent (PM, PCO and SE sites) to high (RO and SF sites), even if 12 sediment samples register a low toxicity and 5 a medium toxicity. The Lido basin is the most investigated one, since it includes the industrial area and the city of Venice: only three sediment samples (one from TE and two from SS) evidence absence of toxicity; 26 sediment samples have a low toxicity, 11 a medium toxicity, 7 a high toxicity and 9 a very high toxicity. Sites at high and very high toxicity are located into or in front of the industrial area. These results evidence that in the Lido basin there are the main evidences of toxic effect for aquatic biota. The Malamocco basin is the less investigated one (5 sites), but seems to be the less toxic one (3 sediment samples with absence of toxicity, 6 with a low toxicity, one with an high toxicity). The Chioggia basin highlights a general medium-high toxicity since 6 sediment samples have a medium toxicity and 5 a high toxicity (a part from two sediment samples with absence of toxicity and two at low toxicity): the pollutants deriving from the town of Chioggia probably affect the sediment of this basin.
Sediments from clam harvesting areas were few investigated (Figure 1). For the A area only the site COA (Coa del latte) can be considered, indicating a low integrated sediment toxicity. In the B area no sampling site for toxicity bioassays is present, even if SS (Sacca Sessola) is located near this area; according to WATI, sediment toxicity in SS ranged from absent to low in the years 2003-2006. Also in the C area no sampling site is present: the nearby site LRI (lago di Rivolta) showed low toxicity. In the D area, the sites CR (Ca' Roman), CH (Chioggia) and VB (Valle di Brenta) are located near this clam harvesting area and evidenced an important toxicity; indeed, CR ranged from absence of toxicity to high toxicity in the years 2003-2005, CH showed an high sediment toxicity and VB a medium sediment toxicity.

site	**sediment sample**	Microtox WholeSediment	*C.gigas* Porewater	sperm_*P.lividus* Porewater	embryo_*P.lividus* Elutriate	*M. galloprovincialis* Elutriate	*C.orientale* WholeSediment	**WATI**	**clam harvesting area**
Palude di Cona	PCO-s03	medium			absent		absent	absent	
Burano	BU-s03	very high			absent		absent	low	
Coa del Latte	COA-s03	medium			medium		absent	low	**A**
Murano	MU-s03	very high			absent		absent	low	
Porto Marghera 46	Z46AB-w05	high						high	
Porto Marghera 47	Z47AB-w05	low						low	
Porto Marghera 46	Z48AB-w05	high						high	
San Giorgio in Alga	GIA-s03	high			medium		absent	medium	
Fusina 1	FU1-s94	low						low	
Fusina 2	FU2-s94	low						low	
Fusina 3	FU3-s94	low						low	
S.Sessola 11	SS11-s94	low						low	
Sacca Sessola	SS-s03	low					absent	absent	**B**
	SS-s05	low			absent		absent	low	
	SS-s04	low			medium		absent	low	
	SS-w04	high			absent		absent	low	
	SS-w05	medium	high	absent	absent	absent	absent	absent	
	SS-wB05	low			medium			low	
Ottagono abbandonato	OTn-s03	high			absent		absent	low	
Malamocco 18	PSI18-s94	low						low	
Valle de Bon	BON-s03	high			low		absent	low	
Lago di Rivolta	LRI-s03	medium			medium		absent	low	**C**
San Pietro in Volta	SPV-s03	high			absent	absent	absent	low	
	SPV-s04	medium			absent		absent	low	
	SPV-s05	medium			absent		absent	absent	
	SPV-w04	medium			absent		absent	absent	
	SPV-w05	high			high	absent	absent	low	
	SPV-wB05	low			absent			absent	
Chioggia 14	PB14-s94	medium						medium	
Petta di Bò	BO-s03	high			medium		absent	medium	
Chioggia 13	PB13-s94	low						low	
Chioggia 12	PB12-s94	low						low	
Ca' Roman	CR-s03	high			medium		absent	medium	
	CR-s04	medium			medium	absent	absent	absent	
	CR-s05	medium			absent		absent	absent	
	CR-w04	high			absent	medium	absent	medium	**D**
	CR-w05	medium			absent	medium	absent	low	
	CR-wB05	high			medium			high	
Chioggia	CH-s03	very high			very high		absent	high	
Valle di Brenta	VB-s03	very high			low		absent	medium	

Table 1. Toxicity classification of sediment samples from the Venice lagoon according to the WATI (Weighted Average Toxicity Index). Investigated sites inside the clam harvesting areas are evidenced.

The Figure 2 shows all the investigated sites for bioaccumulation analyses. In particular we want focus the attention on the evidences from the clam *T. philippinarum* bioaccumulation. Di Domenico et al. (1998) found remarkably increased levels of metals and organic compounds in the clam tissues in the industrial area of Porto Marghera and, second next, in Venice urban environment (Lido basin) respect to the other lagoonal areas (these results were confirmed also by the bioaccumulation in the mussel and in the oyster *Ostrea edulis*). Bortoli et al. (2003) studied specifically butyltins and phenyltins in lagoonal biota and found that clams collected in autumn and winter 1999/2000 showed TBT (tributiltin) concentrations higher than Tolerable Average Residue Level at stations 1BO, 3BO (Lido basin) and 11BO (Chioggia basin, D clam harvesting area), and those of DBT (dibutiltin) at stations 2BO (Lido basin) and 11BO (Chioggia basin, D clam harvesting area). The PCBs concentrations found by Boscolo et al. (2007a) in clams from several rearing areas in December 2003 and in March, June and September 2004, revealed levels below the tolerable daily intake (TDI) suggested by the World Health Organisation. Moreover, the highest total concentration of PCBs was registered in CR2 (Chioggia basin, close to the D clam harvesting area), while the lowest one in MAL2 (Malamocco basin, C clam harvesting area). The clams collected in May 2005 in FU5 (Malamocco basin) and transplanted at CR1 and VB (Chioggia basin, D clam harvesting area) revealed the PAHs elimination in tissues after 30 and 60 days and the bioaccumulation of low-molecular weight PAH compounds after 180 days, particularly in the VB site (Boscolo et al., 2007b).



Chioggia basin				
site	species			
FCL			H	
CBS			H	
7480		T		
SV		T		
7476		T		
4407		T		
4404		T		
4406		T		
4405		T		
CR	M	T		Z
CR1		T		
7475		T		
9BO		T		Z
CH4		T		
CR2		T		
10BO		T		Z
11BO		T		Z
CH	M	T		Z
CH6	M			
7473		T		
7474		T		
VB		T		

Malamocco basin				
site	species			
FU5		T		
8/2B		T		
4410		T		
8BO		T		Z
7483		T		
FCB			H	
7482		T		
8/1B		T		
MAL1		T		
7478		T		
7479		T		
4409		T		
ALB	M			
7477		T		
PEL	M			
L			H	
MAL2		T		
SPV	M			Z

Lido basin									
site	species				site	species			
COA1		T			PLI			H	
AER		T			I			H	
S			H		1BO		T		Z
CMP			H		4411		T		
H			H		CIN	M	T		Z
CEL	M				PMA	M			
CAM	M	T		Z	TE	M	T		Z
5BO		T		Z	TR	M			
G			H		TR1	M			
10	M				CVE	M			
15	M				SS1	M			Z
2	M				GIA1	M			
PSA	M				3B		T		
4BO		T		Z	7484		T		
2BO		T		Z	Q			H	
P			H		6B		T		
SG	M				5/2B		T		
T			H		3BO		T		Z
N			H		FU4	M			
SG1	M			Z	7B		T		
V			H		R			H	
O			H		SS	M	T		Z
U			H		SS2	M			
CSB			H						

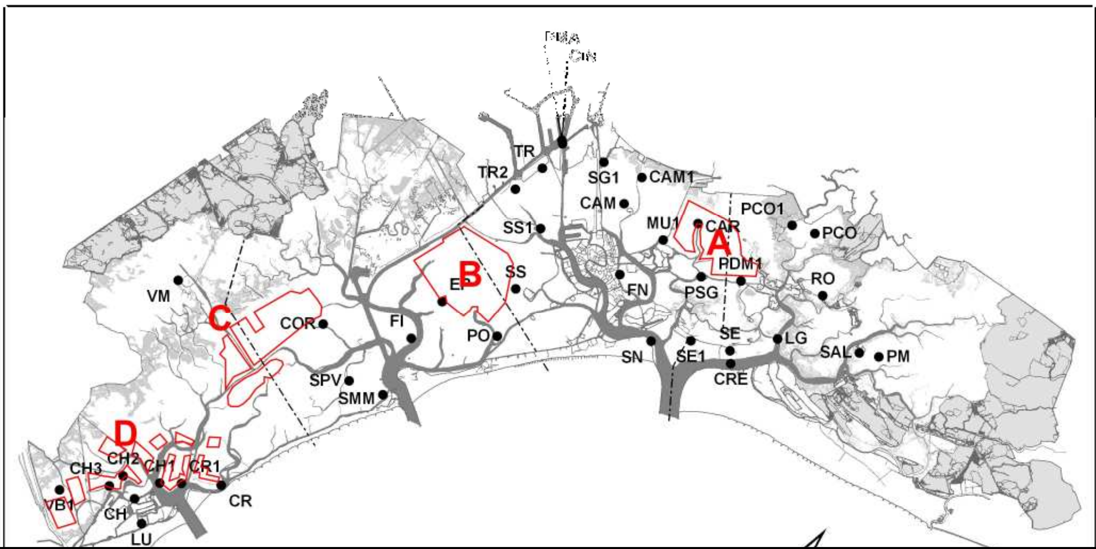
Treporti basin				
site	species			
7BO		T		Z
7481		T		
A			H	
B			H	
M			H	
C			H	
D			H	
E			H	
LG	M			
RDP			H	
CBD			H	
F			H	
TRE	M			
CRE	M			
BUO		T		
SN1	M			

Figure 2. Map of the Venice lagoon indicating the location of the sampling sites for which information on bioaccumulation indicators are available. M=*M.galloprovincialis*; T=*T.philippinarum*; H=*H.diversicolor*; Z=*Z. ophiocephalus*. Red lines in the figure showed the areas for clam harvesting and yellow cells in table evidenced those sites inside the clam harvesting area for which results on clam bioaccumulation are available.

Figure 3 shows the localization of the sites for which peer-reviewed papers report information for the most used couples species-biomarker, that are: *M. galloprovincialis*–adducts, *M. galloprovincialis*–micronuclei, *T. philippinarum*–acetyl cholinesterase (AchE), *T. philippinarum*–vitellogenin, *Nassarius nitidus*-imposex, *Zosterisessor ophiocephalus*-adducts and *Z. ophiocephalus*-EROD.

The most significative evidences on the clam *T. philippinarum* derived from the following studies:

- the measurement of AChE as biomarker of exposure to neurotoxic compounds *T. philippinarum* (Matozzo et al., 2005); a seasonal trend in AChE activity was observed (enzyme activity being higher in January and lower in June) related to both exogenous (pollutant availability) and endogenous factors (physiological status of the animals); a significant reduction in AChE activity was found in clams from TR2, CAM1, PSG (Lido basin) and CH2 (Chioggia basin, D clam harvesting area) (Matozzo et al., 2005).
- The levels of vitellogenin (Vg)-like proteins as biomarkers that detect endocrine alterations; the clams collected at CAM1 and TR2 (Lido basin) had higher Vg-like protein levels, particularly in haemolymph, respect to those collected in sites from Treporti (PCO) and Chioggia (CR1, CH3, LU) basins (Matozzo and Marin 2007).



Treporti basin								
site	species-biomarker							clam area
SAL					N-i			
PCO				T-v				
PCO1			T-ac					
LG		M-m				Z-a	Z-E	
SE		M-m				Z-a		
SE1		M-m			N-i	Z-a		
CRE							Z-E	
PDM1			T-ac					A

Lido basin								
site	species-biomarker							clam area
PSG			T-ac					
CAR					N-i			A
MU1					N-i			
CAM	M-a	M-m				Z-a		
CAM1			T-ac	T-v				
SN					N-i			
SG1							Z-E	
CIN	M-a	M-m				Z-a		
PMA							Z-E	
TE	M-a	M-m				Z-a		
TR2	M-a	M-m	T-ac	T-v		Z-a		
SS1							Z-E	

Malamocco basin								
site	species-biomarker							clam area
PO			T-ac					
EP					N-i			B
FI			T-ac					
SMM					N-i			
SPV						Z-a		
COR			T-ac					C

Chioggia basin								
site	species-biomarker							clam area
CR	M-a	M-m				Z-a		D
CR1				T-v				D
CH	M-a	M-m				Z-a		
CH1			T-ac					D
CH2			T-ac					D
CH3			T-ac	T-v				D
LU			T-ac	T-v				
VB1			T-ac					D

Figure 3. Map of the Venice lagoon indicating the location of the sampling sites for which information with biomarkers by peer-reviewed papers are available. M-a=*M.galloprovincialis*–adducts; M-m=*M. galloprovincialis*–micronuclei; T-ac=*T.philippinarum*–AchE; T-v=*T.philippinarum*–vitellogenin; N-i=*N.nitidus*-imposex; Z-a=*Z.ophiocephalus*-adducts; Z-E=*Z. ophiocephalus*-EROD. Red lines in the figure showed the areas for clam harvesting and yellow cells in table evidenced those sites inside the clam harvesting area for which results on clam biomarkers are available.

CONCLUSIONS

The ecotoxicological studies performed in the Venice lagoon can be used to determine the quality of the authorized clam harvesting areas. The A, B and C areas showed low sediment toxicity and did not evidence high biomarker and bioaccumulation levels in the clam *T. philippinarum*. At the contrary, the D area showed sediments that reach medium and high toxicity levels, a significative reduction of acetylcholinesterase levels in clams (denoting exposure to neurotoxic compounds) and high levels of clam bioaccumulation of organic compounds (TBT and PCB).

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