POPs BIOACCUMULATION IN BOTTOM-MUSSELS FROM THE GULF OF TARANTO (IONIAN SEA, ITALY) COLLECTED IN SEMIENCLOSED SEAWATER BASINS NEAR URBAN AND INDUSTRIAL POLLUTION SOURCES

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Introduction

The mainland used by Taranto industrial area and the bottom of two semienclosed seawater basins, Mar Grande and Mar Piccolo, are part of one of the largest italian remediation sites as listed by national legislation aimed at the identification and rehabilitation of polluted areas.[1] Local Environment Agencies are involved at several stages in the remediation process and, of particular relevance for the present paper, they are responsible for the validation of results produced by laboratories contracted by the parties responsible for individual areas. One of the several data validation included the duplicate laboratory analysis for the determination of PCDD/Fs, dioxin-like PCBs, and total PCBs for 32 bottom-mussel samples with the aim of assessing the health of the seabed in relation with the pollutants discharged into local water basins by industrial, military an civil sources over the past decades.

The Taranto area (Southern Italy) hosts several industrial facilities including thermal/combustion processes[2] with remarkably high raw materials and high energy demand and known potential sources of PCDD/Fs and PCBs release to air, land, and water.[3] These facilities include a large integrated steel plant, a medium-sized oil refinery, a large cement-works, two power plants, and three waste incinerators as well as a large naval base with military shipyards. Taranto is a relatively large southern Italian city, the capital of the Province of Taranto and an important commercial port, with a population of over two-hundred thousands inhabitants.

The magnitude of the environmental pressure on the Taranto marine environment is known to some extent.[4,5] Although there is some information on the potential impact of the measured environmental levels of heavy metals on the food chain through molluscs reared in Taranto coastal area,[6,7] no data are available on presence of PCDD/F and dioxin-like PCBs in filter-feeding organisms at direct or close proximity with polluted sediments, like bottom-mussels, despite the presence of numerous rope-mussel culture installations.

Materials and methods

Sampling. A total of 81 sampling stations on an ideal 450x450m grid were selected according to their relevance for the five areas of rope-mussel cultures (identified as A, B, C, D, E) as depicted in figure 1 (map courtesy of ICRAM-ISPRA). 32 of those stations were analysed in duplicate for validation.

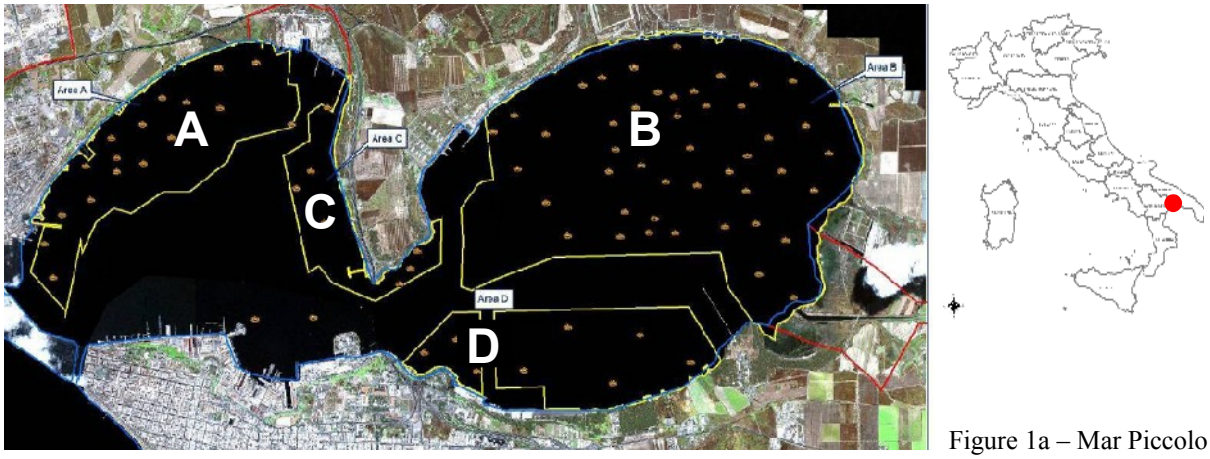

Figure 1a – Mar Piccolo

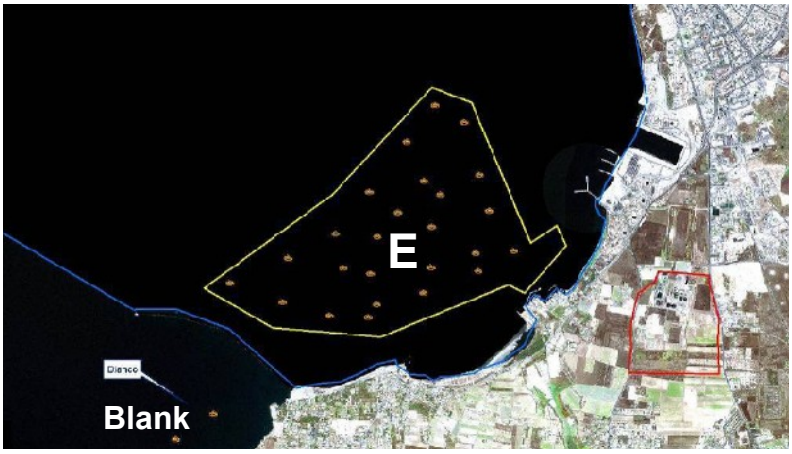

Figure 1b – Mar Grande

Mussels were collected according a pre-defined sampling protocol that is widely used in Italy at every marine remediation site. For every sampling station, a minimum of 30 molluscs of similar length, approximatively between 70% and 90% of the maximum length of the sampled population, were collected. Dive-sampling operations were carried out from 21st to 23rd July 2009. On-site, whole mussels were rinsed with sea water and wrapped in wet tissues before being transported to the laboratory while maintaining a temperature of 4-6 C deg. Laboratory pre-analysis operations included biometric measurements (length) as well as the separation of the soft tissue from the shell and the forming of three pools that were homogenised and frozen for storage.

HRMS Analysis of mussel tissues. Tissue samples were freeze-dried under vacuum (lyophilised) prior to extraction by Accelerated Liquid Extraction (Dionex, Sunnyvale CA USA) with Hexane/Acetone 3:2. Fat content was determined although final concentrations are expressed on a wet basis according to EU Regulation 1881/2006. The extracts were percolated through diatomaceous earth treated with concentrated sulphuric acid for fat elimination and subsequently purified by means of an automated clean-up process with a PowerPrep system (Fluid Management System, Waltham, Massachusetts) using disposable columns (multilayer silica, alumina and carbon). Labelled standards were purchased from Cambridge Isotope Laboratories (Andover MA USA) and used according to Method EPA 1613 for PCDD/Fs and EPA 1668 for PCBs. PCDD/Fs were separated by high resolution gas chromatography (HRGC) on a DB-5 MS capillary column (60 m x 0.25 mm, 0.25 μm film thickness, J&W Scientific, California). Isotope-dilution high-resolution mass-spectrometry determinations (HRMS) were carried out on a DFS High Resolution system (Thermo Fisher, Bremen, Germany) at a resolution of 10000 operating with electron ionisation (EI) at 45 eV in the selected ion monitoring (SIM) mode. PCBs and dl-PCBs were separated by HRGC on a DB-5 MS capillary column (30 m x 0.25 mm, 0.25 μm film thickness, J&W Scientific, California) and determined by HRMS, in the same operating conditions used for PCDD/Fs. For each batch of seven samples a laboratory blank and a control sample were analysed.
Toxic equivalent (TEQ) values were calculated using OMS Toxic Equivalency Factors (WHO-TEFs, 1998) and expressed as upper-bound concentrations.

Results and discussion
POPs and heavy metals bioaccumulation studies in marine organisms as molluscs and necto-bentonic species have been performed in several italian remediation sites as a preliminary assessment before sediment sampling and chemical analyses were started. The magnitude (or lack) of bioaccumulation was then used to steer the sampling effort, in both numerousness and array of chemical species analysed, towards the most critical areas as identified by the more polluted molluscs.
Table 1 presents the results of the PCDD/Fs and PCBs determinations for mussels (*Mytilus Galloprovincialis*) collected on the bottom of the five A, B, C, D, E areas shown in figure 1. Two sampling stations outside the areas of rope-mussel culture were selected as Blank sampling stations. The bottom-mussels results represent an indicator of ecosystem health and is not directly related to rope-mussels consumption by the general population.

Sample number	*Zone*	*PCDD/F*	*PCB*	*TEQ tot*
		pg WHO-TE/g wet weight		
1	A	2,33	11,69	14,02
2	A	2,33	11,32	13,65
3	A	2,48	10,48	12,96
4	A	2,07	10,59	12,66
5	A	1,85	12,04	13,89
6	B	0,73	1,55	2,28
7	B	1,34	2,14	3,48
8	B	1,62	2,87	4,49
9	B	1,42	2,18	3,6
10	B	0,86	2,76	3,62
11	B	0,77	2,22	2,99
12	B	0,75	2,14	2,89
13	B	0,57	1,68	2,25
14	B	0,85	2,62	3,47
15	B	0,77	1,96	2,73
16	B	0,95	2,76	3,71
17	B	0,64	2,54	3,18
18	B	0,64	1,89	2,53
19	C	2,25	9,03	11,28
20	C	1,07	9,55	10,62
21	D	2,03	4,67	6,7
22	D	0,86	4,07	4,93
23	D	1,36	12,59	13,95
24	Blank area	0,69	4,9	5,59
25	E	0,48	5,13	5,61
26	E	0,77	5,82	6,59
27	E	0,46	4,87	5,33
28	E	0,57	4,48	5,05
29	E	0,37	3,9	4,27
30	E	0,36	5,23	5,59
31	E	0,36	4,56	4,92
32	E	0,27	2,36	2,63

Results are expressed as wet weight concentrations as required by relevant legislation for fish food products in the EU (Regulation 1881/2006). Average fat content was about 1.2%. PCDD/Fs concentrations ranged from 0.27 to 2.48 pgWHO-TE/g and the lowest values were found for mussels collected in Zone E (mean 0,46 pgWHO-TE/g) while highest values were found for mussels from Zones A (mean 2.21 pgWHO-TE/g) and C (mean 1.66 pgWHO-TE/g). Dioxin-like PCBs ranged from 1.55 to 12.59 pgWHO-TE/g with lowest values found for mussels collected in Zone B (mean 2.25 pgWHO-TE/g) while highest values were found for mussels from Zones A (mean 11.22 pgWHO-TE/g), C (mean 9.29 pgWHO-TE/g) and D (mean 7.11 pgWHO-TE/g). It has to be noted that the dl-PCBs level for the blank sampling station (Figure 1b) is quite high compared to the lowest values found in zone B, and are more similar to what is found for Zone E, to which the blank stations are actually not at very high distance. The total WHO-TEQ values very much reflect the same spatial distribution of dl-PCB, since they are the main contributors to total toxicity. A similar pattern was found for terrestrial animals from farms close to the Taranto industrial area[7] and a general predominance of dl-PCB is actually a trend that has been often observed for the fish food group.[8] Total WHO-TEQ ranged from 2.25 to 14.02 pgWHO-TE/g with lowest values in Zone B (mean 3.17 pgWHO-TE/g) while highest values were found for mussels from Zone

A (mean 11.22 pgWHO-TE/g). A more detailed evaluation of contributors to toxicity and preliminary source apportionment can be obtained by looking at PCDD/F:dl-PCB ratio. For Zone A, whose bottom-mussels are the most contaminated, a 1:5 ratio is observed, while for the less contaminated area (Zone B) a 1:2.5 ratio is found. Samples collected in Zones C and D appear to be somewhat less homogeneous. An interesting case is Zone E, whose PCDD/Fs values are the lowest (mean 0.46 pg WHO-TE/g) while dl-PCBs remain quite high with a mean value of 4.54 pg WHO-TE/g, resulting in an average 1:10 ratio. This result could suggest that while Zone E shows the lowest PCDD/Fs values because it is the most segregated from the PCDD/F sources located in the industrial area (that is in the upper left corner of Figure 1a), a significant source of PCBs (like the discharge of dielectric fluid) could have been in the nearby. Congener profile for sample 5 Zone A, the most contaminated, is shown in Figure 2 and presents a PCDD/Fs profile with 2378-TCDF as the most abundant congener, that is consistent with the known more pronounced bioaccumulation for congeners with the least chlorine atoms.[9]

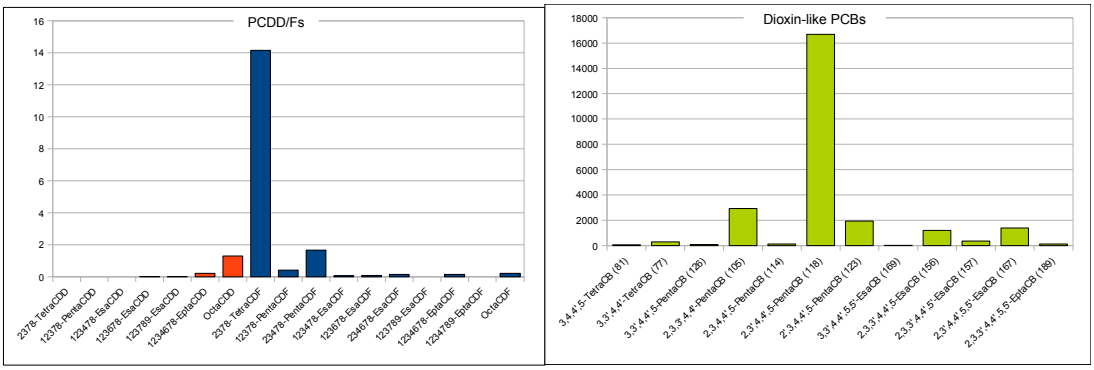

Figure 2

Conclusions

POPs bioaccumulation is a useful tool in assessing the ecological health status of the marine ecosystem. For the area under investigation, the Gulf of Taranto, Zone A appears to be the most contaminated with highest values for both PCDD/Fs and PCBs and this appears to be consistent with the proximity of the 5-decades old industrial area, that operated well before the ban on PCBs use entered into force (late 1970) and well before restrictions in the discharge of industrial waste to water basins were promulgated (mid 1970). Present releases to water could include washout of contaminated soil as well as continuing percolation from discharge to inadequate landfills. Actions to reduce those emissions to water should be undertaken in order to decrease PCDD/Fs and PCBs levels in the marine environment. As a continuing effort to monitor the insurgence of any potential risk related to the food-chain, the local Health Authority has communicated that all samples of reared rope-mussels collected during their Official Control activities have so far shown POPs levels within EU legal limits (Reg 1881/2006).

Acknowledgements

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