Ann Ist Super Sanità 2020 | Vol. 56, No. 4: 452-461
DOI: 10.4415/ANN_20_04_07

ORIGINAL ARTICLES AND REVIEWS

Dioxins and PCBs contamination in mussels from Taranto (Ionian Sea, Southern Italy): a seven years spatio-temporal monitoring study

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Abstract

Introduction. Taranto is of particular Public Health relevance for the presence of industrial sources of dioxins and PCBs. The aim of this study was to monitor these pollutants in mussels produced from 2012 to 2018.

Materials and methods. Mussels were collected on a monthly basis with random sampling. Concentrations were determined through accredited methods.

Results. 622 samples were collected. Dioxins and PCBs showed higher median concentrations in Mar Piccolo 1st Inlet (p_s<0.0001; Dioxins: 1.43 pg WHO-TEQ/g ww; Dioxins+DL-PCBs: 5.98 pg WHO-TEQ/g ww; DL-PCBs: 4.57 pg WHO-TEQ/g ww; NDL-PCBs: 61.54 ng/g ww) and in III trimester for all basins (p_s<0.02). In Mar Piccolo 1st Inlet, there was a linear increase of dioxins and PCBs moving North (p_s<0.05).

Conclusions. These findings confirmed higher concentrations in Mar Piccolo 1st Inlet during the summer period and supported the validity of the Public Health measures adopted by the Department of Prevention of Taranto.

Key words
- dioxins
- PCBs
- environmental contamination
- Taranto
- mussel

INTRODUCTION

Polychlorinated dibenzo-p-dioxins (PCDD), polychlorinated dibenzofurans (PCDF) and polychlorinated biphenyls (PCBs) are classified by WHO as environmental pollutants with a global distribution and high resistance to degradation [1-6].

Long-term exposure to dioxins (PCDD/Fs) and some PCBs, referred to as dioxin-like PCBs (DL-PCBs) due to their similar toxicological properties, has been shown to cause a range of adverse effects on the nervous, immune and endocrine systems, to impair reproductive function and to cause cancer. Other PCBs referred to as non-dioxin-like PCBs (NDL-PCBs) have a different mechanism of toxicity, but they too can seriously damage human health [1, 3, 5-9].

More than 80% of total exposure is attributable to dietary intake, that represents the main route of PCDD/Fs and PCBs exposure for humans [1, 5, 7]. In particular, the consumption of animal origin foods, like milk, meat, eggs, leads to a greater risk of bioaccumulation due to the lipophilic properties of these pollutants [1, 5].

Taranto, a coastal city in the South of Italy (Ionian Sea, Puglia Region), is of particular relevance in this context due to the type of industrial settlements accounting for known potential sources of PCDD/Fs and PCBs (the most important steel plant in Europe, an oil refinery, a cement works, thermoelectric plants, waste incinerators, discharges and military harbours) and to the environmental contamination present in different matrices, including marine sediments - in particular in Mar Piccolo 1st Inlet [10-14]. In this regard, the city is surrounded by two basins, the Mar Grande (a wide roadstead with maximum depth of about 35 meters and a mean salinity of 37.8‰) and the Mar Piccolo (a inner basin with lagoon features, divided into two inlets with a maximum depth of 13 and 8 meters, respectively, and a mean salinity of 35.1‰), that are home to several mussel (*Mytilus galloprovincialis*, Lamarck 1819) culture areas which account for a significant production at national level [13, 14].

Mussels can represent a food at risk of contamination, because are filter-feeding organisms with high bio-

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accumulation and low biotransformation potential for both organic and inorganic contaminants [14-16].

Since 2011, the Department of Prevention of the Local Health Authority of Taranto has carried out a monitoring plan of in order to assess PCDD/Fs and PCBs contamination in *M. galloprovincialis* from Mar Piccolo and Mar Grande. The results of a study on mussel samples collected between March and December 2011 [14] as well as of the activities carried out in these years by the Department of Prevention of the Local Health Authority of Taranto, showed results of non-compliance with European legislation [16, 17] as regards the levels of PCDD/Fs and DL-PCBs in the 1st Inlet of the Mar Piccolo during the summer period. This led the Department of Prevention of the Local Health Authority of Taranto to propose to Puglia Region the adoption of the Regional Ordinance n. 188/2016 to block the removal and handling of commercial mussels in Mar Piccolo 1st Inlet, with the only possibility of moving juvenile mussels (<5 cm) from Mar Piccolo 1st Inlet to the other basins by March 31, after a sampling result in accordance with EU standards for dioxins and PCBs [16, 17]. In September 2018, the Puglia Region published an update of the Ordinance (n. 532/2018), anticipating the temporal limit to February 28, in order to account for an increase in dioxins/PCBs mussels contamination found starting from the II trimester.

The aim of this study was to update those evidences through the monitoring of the content and the spatio-temporal distribution of PCDD/Fs, DL-PCBs and NDL-PCBs in mussels produced from January 2012 to December 2018 in all the mussel farming plants of the Mar Grande and Mar Piccolo of Taranto, in order to guarantee the healthiness of the product placed on the market, identify marine areas and critical seasons for mussel contamination, and verify and develop effective public health strategies to protect the health of consumers together with the production chain of the territory.

MATERIALS AND METHODS

Sampling

We included in this study the mussel samples (*M. Galloprovincialis*) collected by the staff of the Department of Prevention of Local Health Authority of Taranto between January 2012 and December 2018 in Mar Piccolo and Mar Grande. For each sample, numerous aliquots of product (at least 1 kg total) were taken from various points between 0 and 4 meters deep, in order to obtain a representative sample of any different levels of contamination present in the mussels both in contact with sediments or in aquatic suspension. Mussels were collected on a monthly basis with random sampling using predefined square grid cells within the three basins. The geographic coordinates of the sampling areas were established from the georeferencing maps created by ISPRA (National Institute for Environmental Protection and Research), taking into account the level of contamination of the sediments. The samples were sent for chemical analysis to the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale" (National Reference Laboratory for Halogenated POPs in food and feed).

Chemical analysis

Chemicals

Solvents such as n-hexane, dichloromethane, acetone, toluene and isooctane were organic residue analysis quality (Honeywell Burdick & Jackson, Seezle, Germany). Ultra-pure water was generated within the laboratory by means Purelab option-Q system (ELGA LabWater, High Wycombe, United Kingdom). Other reagents included anhydrous sodium sulphate, concentrated sulphuric acid and sodium chloride, all at reagent grade (Honeywell Burdick & Jackson, Seezle, Germany).

Prepacked multilayer silica, alumina, and carbon columns were obtained from Fluid Management Systems (Massachussetts, USA).

All standard solutions were supplied by Wellington Laboratories (Guelph, Ontario, Canada). Calibration solutions DF-CVS (CS1 through CS4), $^{13}C_{12}$-labeled internal standard DF-LCS-C200, and recovery standard DF-IS-J were used for PCDD/Fs analysis. Calibration solutions WP-CVS (CS1 through CS7), $^{13}C_{12}$-labeled internal standard WP-LCS, and recovery standard P48-RS-STK were prepared for DL-PCB analysis. Calibration solutions P48-M-CVS (CS1 through CS5), $^{13}C_{12}$-labeled internal standard P48-M-ES, and recovery standard P48-RS-STK were selected for NDL-PCB analysis.

Analytical methodology

The 17 PCDD/Fs, the 12 DL-PCBs and the 6 indicator NDL-PCBs [16, 17] were determined through accredited methods in accordance with ISO EN 17025, based on US EPA (1994) Method 1613 B for PCDD/Fs and US EPA (2008) Method 1668 B for PCBs. Both methods are based on isotopic dilution and high resolution mass spectrometry (HRMS) detection. In order to adapt the analytical procedures to the matrix under examination, variations have been made in the extraction and purification phases of the sample.

All the samples under examination were homogenized by a knife mill Grindomix GM-200 (Retsch, Dusseldorf, Germany) and a representative rate of approximately 5±2 g was taken from them. The samples were dried for at least 8 hours in an oven at a temperature of 40±5°C and, before extraction, were mixed with anhydrous sodium sulphate in a ratio of 1:3 (w/w) and fortified with a mixture of the internal standards containing: 17 PCDD/Fs $^{13}C_{12}$-labeled (0.2-0.4 ng); 12 DL-PCBs $^{13}C_{12}$-labeled (1.0 ng); 6 NDL-PCBs $^{13}C_{12}$-labeled (2.0 ng).

The samples were extracted by accelerated solvent extraction with n-hexane-acetone (80:20, v/v) using a Dionex ASE 350 (Thermo Fisher Scientific, Waltham, MA) at 1,500 psi and 125°C.

The extract, collected in a glass vial, was filtered on anhydrous sodium sulphate and collected in a volumetric flask, dried on a rotary evaporator with a water bath at 40±5°C.

After solvent evaporation, the extract was dissolved in hexane and subjected to liquid-liquid partitioning with concentrated sulfuric acid, 20% aqueous potassium hydroxide, and saturated aqueous sodium chloride.

Subsequently, the extract was purified, in sequence, on chromatographic columns of multilayer silica gel, alumina and carbon, using an automated clean-up process with a Power-Prep™ system (FMS, Massachusetts, USA).

The two eluates containing PCDD/Fs and PCBs, were concentrated by evaporation in nitrogen stream and dissolved in the corresponding recovery standards solutions ($^{13}C_{12}$-labeled PCDD/Fs and PCBs different from the previous ones).

The instrumental analysis was performed using high resolution gas chromatography - high resolution mass spectrometry (HRGC-HRMS), using GC Trace Series 2000 coupled to a MAT 95 XL (Thermo Fisher Scientific, USA) and a Trace Series 1310 GC, coupled to a DFS (Thermo Fisher Scientific, USA). The chromatographic separation of the 17 PCDD/Fs was carried out on a DB-5 MS capillary column 60m x 0.25mm x 0.10µm (J&W Scientific, California, USA). The chromatographic separation of DL-PCBs and NDL-PCBs was carried out on HT8-PCB capillary column 60m x 0.25mm x 0.25µm (SGE Analytical science, Melbourne, Australia). The acquisition of the masses was carried out in Single Ion Monitoring (SIM) mode at a resolution of 10,000, selecting the masses indicated by the method. As regards 17 PCDD/Fs and 12 DL-PCBs, TEQ concentrations were determined by multiplying the analytical result of each congener by the corresponding WHO-TEF, while for NDL-PCBs, the result was reported as the sum of the 6 indicator congeners. All values have been reported as upper bound concentrations, that is all values below the limit of quantification (LOQ) are supposed to be equal to the respective LOQ.

A laboratory blank and a control sample were analyzed for each batch of 10 and 20 samples, respectively. Recovery rates of labeled congeners ranged from 60% to 90%, and the analytical uncertainty was in the order of ±18% for WHO-TEQs and the sum of six NDL-PCBs. Method performance was in agreement with the requirements for method of analysis used in official control of the levels of PCDD/Fs and PCBs in foodstuff [16, 17] and has been successfully verified in many proficiency tests over 15 years.

Statistical analysis

Statistical analysis was performed using R version 3.6.2 (released on 2019-12-12). Statistical significance α was fixed to 0.05. Maps were created with Microsoft Excel version 2002 (Build 12527.20194).

In order to account for non-normality, evaluated through Shapiro-Wilk test, numerical variables (means of the measured values of the four pollutants concentrations) [16, 17] were reported as median and IQR and compared first through Kruskal Wallis rank sum test and then through pairwise Wilcoxon rank sum test with Benjamini & Hochberg correction for p values (False Discovery Rate). Comparisons were carried out between basins (on the overall sample) and between trimesters (separately for each of the three basin). For each basin and pollutant (values), a multivariable linear median regression model (linear quantile regression with τ=0.5) was then fitted in order to assess the effect of latitude (hundredths of °N) and longitude (hundredths of °E) on the median of each pollutant values. The algorithmic method used to compute the fit was the modified version of the Barrodale and Roberts algorithm. Standard errors were computed through a Huber sandwich estimate using a local estimate of the sparsity. β coefficients can be interpretable as the increase in pollutant values median (median difference) for an increase of one hundredth of °N or °E. In order to assess the pairwise correlation between the four pollutants (values) non-normally distributed, Spearman rank correlation coefficient ρ was calculated for each basin and combination. P-values were computed via the asymptotic t approximation.

Categorical variables (means of the measured values of the three pollutant concentration minus the associated expanded uncertainty that are above the established EU maximum level) [16, 17] were reported as absolute and relative frequencies and, in order to account for low expected frequencies (n<5), were compared first through Fisher Exact test (Fisher-Freeman-Halton Exact Test for contingency tables larger than 2x2) and then through pairwise Fisher Exact test with Benjamini & Hochberg correction for p values (False Discovery Rate). Comparisons were carried out between basins (on the overall sample) and between trimesters (separately for each of the three basin). For each basin and pollutant (values above EU maximum level) with sufficient frequencies (n>30), a multivariable binary logistic regression model was then fitted in order to assess the effect of latitude (hundredths of °N) and longitude (hundredths of °E) on the odds of pollutant value above the EU maximum level. Odds Ratio can be interpretable as the increase in the odds of pollutant value above the EU maximum level for an increase of one hundredth of °N or °E. In order to assess the pairwise correlation between pollutants values above the EU maximum level, Pearson correlation coefficient ϕ was calculated for each combination in Mar Piccolo 1st Inlet. P-values were computed via t distribution.

RESULTS

622 mussel samples were collected between 2012 and 2018, 208 in Mar Piccolo 1st Inlet, 207 in Mar Piccolo 2nd Inlet and 207 in Mar Grande.

Spatial distribution of the pollutants values between the three basins was shown in the left side of *Figure 1*. Results of overall pollutants values distribution and comparisons between basins were reported in *Table 1*. Kruskal Wallis rank sum test showed a significant difference between basins for all pollutants (p_s<0.0001). Pairwise Wilcoxon rank sum test showed: higher values in Mar Piccolo 1st Inlet compared both to Mar Piccolo 2nd Inlet and Mar Grande for all pollutants (p_s<0.0001); higher values in Mar Piccolo 2nd Inlet compared to Mar Grande for dioxins and NDL-PCBs (p_s<0.0001); lower values in Mar Piccolo 2nd Inlet compared to Mar Grande for DL-PCBs (p=0.0067).

Results of Mar Piccolo 1st Inlet pollutants values distribution and comparisons between trimesters were reported in *Table 2*. Kruskal Wallis rank sum test showed

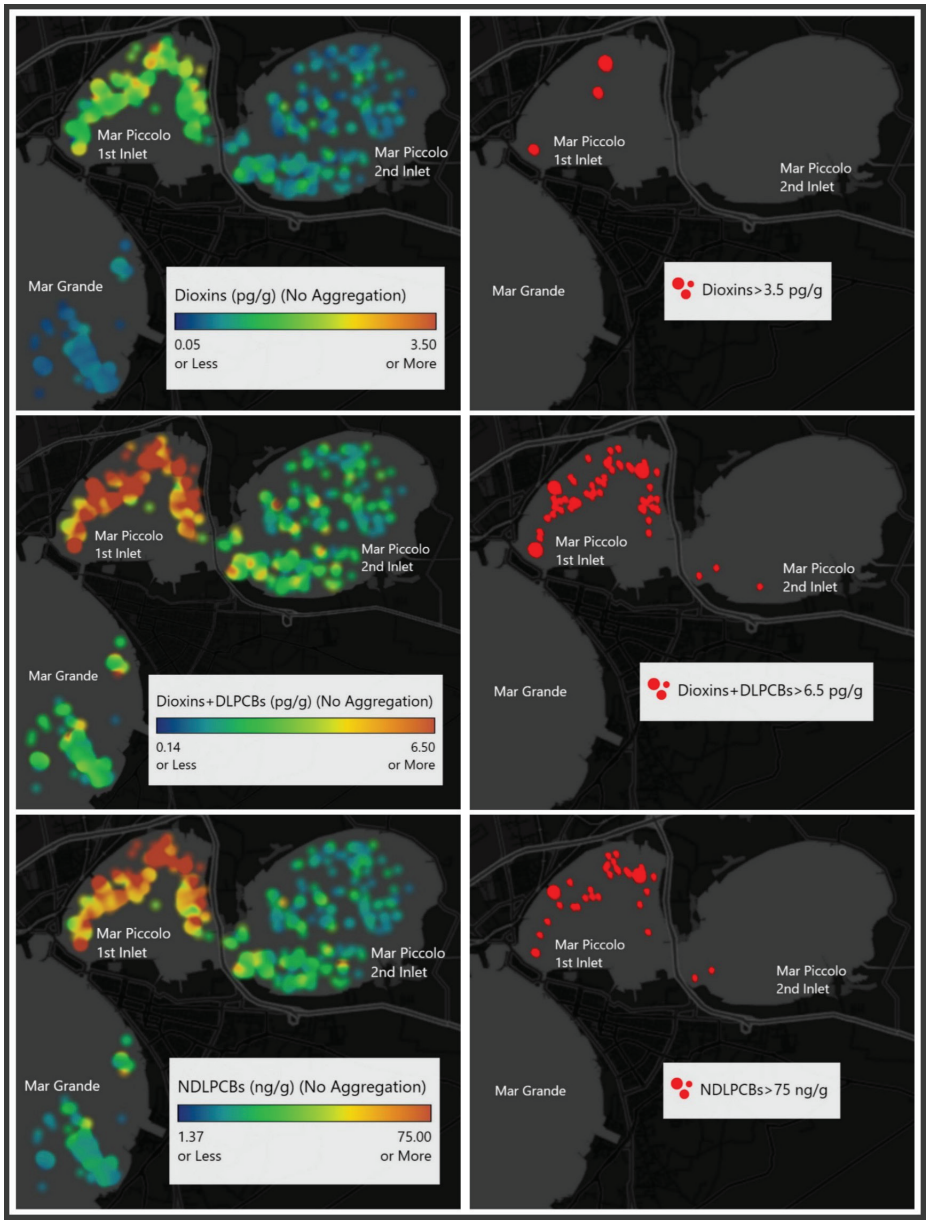

Figure 1
Spatial distribution of mussel pollutants (values and values above EU maximum level) between and within the three basins.
Dioxins: sum of dioxins (WHO-PCDD/F-TEQ); DLPCBs: sum of dioxin-like PCBs (WHO-PCB-TEQ); dioxins+DLPCBs: sum of dioxins and dioxin-like PCBSs (WHO-PCDD/F-PCB-TEQ); NDLPCBs: sum of non-dioxin like PCBs: PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180 (ICES – 6); pollutants on the left: mean of the measured values, wet weight; pollutants on the right: mean of the measured values minus the expanded uncertainty of the mean, wet weight [16, 17].

a significant difference between trimesters for all pollutants (p_s<0.0005). Pairwise Wilcoxon rank sum test showed: lower values of dioxins in both trimesters I and II compared both to III and IV (p_s<0.004); differences in Dioxins+DL-PCBs and DL-PCBs between all trimesters with higher values in trimester III (p_s<0.03); except for II-IV comparison (p=0.071), lower values of NDL-PCBs in both trimesters I and IV compared both to II and III (p_s<0.02).

Results of Mar Piccolo 2nd Inlet pollutants values distribution and comparisons between trimesters were reported in *Table 2*. Kruskal Wallis rank sum test showed a significant difference between trimesters for all pollutants (p_s<0.0001). Pairwise Wilcoxon rank sum test showed: lower values of dioxins and NDL-PCBs in trimesters I, II and IV compared to III (p_s<0.002); except for I-IV comparisons (p_s>0.05), differences in Dioxins+DL-PCBs and DL-PCBs between all trimesters with higher values in trimester III (p_s<0.003).

Results of Mar Grande pollutants values distribution and comparisons between trimesters were reported in *Table 2*. Kruskal Wallis rank sum test showed a significant difference between trimesters for all pollutants (p_s<0.0006). Pairwise Wilcoxon rank sum test showed:

Table 1
Mussel pollutants values distribution between basins (overall), Kruskal Wallis rank sum test and Pairwise Wilcoxon rank sum test (Benjamini-Hochberg correction)

2012-18	Dioxins (pg/g)		Dioxins+DLPCBs (pg/g)		DLPCBs (pg/g)		NDLPCBs (ng/g)	
Overall (n=622)	**Median (IQR)**	**p**	**Median (IQR)**	**p**	**Median (IQR)**	**p**	**Median (IQR)**	**p**
Basin		**<.0001**		**<.0001**		**<.0001**		**<.0001**
Mar Piccolo 1st Inlet (n=208)	1.43 (0.98)		5.98 (4.20)		4.57 (3.78)		61.54 (35.06)	
Mar Piccolo 2nd Inlet (n=207)	0.63 (0.41)		2.00 (1.29)		1.34 (0.96)		20.05 (12.79)	
Mar Grande (n=207)	0.41 (0.28)		2.00 (1.74)		1.61 (1.67)		16.24 (9.67)	
Basin (pairwise)								
Mar Piccolo 1st Inlet - Mar Piccolo 2nd Inlet		**<.0001**		**<.0001**		**<.0001**		**<.0001**
Mar Piccolo 1st Inlet - Mar Grande		**<.0001**		**<.0001**		**<.0001**		**<.0001**
Mar Piccolo 2nd Inlet - Mar Grande		**<.0001**		.56		**.0067**		**<.0001**

Dioxins: sum of dioxins (WHO-PCDD/F-TEQ); DLPCBs: sum of dioxin-like PCBs (WHO-PCB-TEQ); dioxins+DLPCBs: sum of dioxins and dioxin-like PCBSs (WHO-PCDD/F-PCB-TEQ); NDLPCBs: sum of non-dioxin like PCBs: PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180 (ICES – 6); all pollutants: mean of the measured values, wet weight [16, 17].

Table 2
Mussel pollutants values distribution between trimesters (Mar Piccolo 1st Inlet, Mar Piccolo 2nd Inlet and Mar Grande), Kruskal Wallis rank sum test and Pairwise Wilcoxon rank sum test (Benjamini-Hochberg correction)

2012-18	Dioxins (pg/g)		Dioxins+DLPCBs (pg/g)		DLPCBs (pg/g)		NDLPCBs (ng/g)	
Mar Piccolo 1st Inlet (n=208)	**Median (IQR)**	**p**	**Median (IQR)**	**p**	**Median (IQR)**	**p**	**Median (IQR)**	**p**
Trimester		**<.0001**		**<.0001**		**<.0001**		**.0004**
I (n=56)	1.13 (0.63)		4.05 (2.19)		2.98 (2.28)		50.35 (28.74)	
II (n=62)	1.15 (1.02)		7.40 (4.30)		5.98 (3.43)		70.33 (35.38)	
III (n=42)	1.97 (1.17)		9.63 (4.24)		7.92 (3.54)		74.21 (35.74)	
IV (n=48)	1.60 (0.81)		5.26 (2.76)		3.61 (2.06)		59.87 (24.73)	
Trimester (pairwise)								
I - II		.58		**<.0001**		**<.0001**		**.0060**
I - III		**<.0001**		**<.0001**		**<.0001**		**.0029**
I - IV		**.0001**		**.0031**		**.024**		.098
II - III		**.0001**		**.0006**		**.0043**		.44
II - IV		**.0038**		**.0040**		**.0001**		.071
III - IV		.060		**<.0001**		**<.0001**		**.010**
Mar Piccolo 2nd Inlet (n=207)	**Median (IQR)**	**p**	**Median (IQR)**	**p**	**Median (IQR)**	**p**	**Median (IQR)**	**p**
Trimester		**<.0001**		**<.0001**		**<.0001**		**<.0001**
I (n=57)	0.55 (0.28)		1.50 (0.75)		0.96 (0.45)		17.57 (11.41)	
II (n=63)	0.60 (0.48)		2.23 (1.21)		1.58 (0.79)		20.94 (9.50)	
III (n=40)	0.91 (0.64)		3.81 (3.68)		2.88 (2.72)		27.14 (26.40)	
IV (n=47)	0.59 (0.22)		1.80 (0.90)		1.11 (0.64)		19.31 (9.82)	
Trimester (pairwise)								
I - II		.46		**<.0001**		**<.0001**		.059
I - III		**<.0001**		**<.0001**		**<.0001**		**<.0001**
I - IV		.21		.18		.34		.46
II - III		**<.0001**		**<.0001**		**<.0001**		**.0019**
II - IV		.78		**.0022**		**<.0001**		.23
III - IV		**<.0001**		**<.0001**		**<.0001**		**.0003**

Continues

Table 2
Continued

Mar Grande (n=207)	Median (IQR)	p	Median (IQR)	p	Median (IQR)	p	Median (IQR)	p
Trimester		**<.0001**		**<.0001**		**<.0001**		**.0005**
I (n=58)	0.42 (0.19)		1.42 (0.57)		0.97 (0.50)		14.38 (6.04)	
II (n=59)	0.27 (0.13)		2.37 (1.32)		2.08 (1.30)		14.75 (7.13)	
III (n=45)	0.61 (0.29)		3.74 (1.66)		3.19 (1.68)		22.07 (9.63)	
IV (n=45)	0.45 (0.26)		1.74 (0.96)		1.26 (0.83)		16.24 (9.30)	
Trimester (pairwise)								
I - II		**<.0001**		**<.0001**		**<.0001**		.35
I - III		**<.0001**		**<.0001**		**<.0001**		**.0005**
I - IV		.18		**.0093**		**.0019**		.35
II - III		**<.0001**		**<.0001**		**<.0001**		**.0021**
II - IV		**<.0001**		**.0078**		**.0002**		.79
III - IV		**.0056**		**<.0001**		**<.0001**		**.015**

Dioxins: sum of dioxins (WHO-PCDD/F-TEQ); DLPCBs: sum of dioxin-like PCBs (WHO-PCB-TEQ); dioxins+DLPCBs: sum of dioxins and dioxin-like PCBSs (WHO-PCDD/F-PCB-TEQ); NDLPCBs: sum of non-dioxin like PCBs: PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180 (ICES – 6); all pollutants: mean of the measured values, wet weight [16, 17].

Table 3
Multiple linear median regression for each basin and mussel pollutant (values)

2012-18	Dioxins (pg/g)		Dioxins+DLPCBs (pg/g)		DLPCBs (pg/g)		NDLPCBs (ng/g)	
Mar Piccolo 1st Inlet (n=208)	**β (95%CI)**	**p**	**β (95%CI)**	**p**	**β (95%CI)**	**p**	**β (95%CI)**	**p**
Latitude ($_{hundredth}$°N)	0.31 (0.01;0.61)	**.042**	1.60 (0.42;2.78)	**.0082**	1.43 (0.34;2.52)	**.011**	16.67 (5.89;27.46)	**.0026**
Longitude ($_{hundredth}$°E)	0.00 (-0.12;0.12)	.99	-0.34 (-0.84;0.16)	.19	-0.22 (-0.67;0.23)	.33	-3.01 (-7.36,1.34)	.17
Mar Piccolo 2nd Inlet (n=207)	**β (95%CI)**	**p**	**β (95%CI)**	**p**	**β (95%CI)**	**p**	**β (95%CI)**	**p**
Latitude ($_{hundredth}$°N)	-0.04 (-0.12;0.04)	.34	-0.26 (-0.50;-0.02)	**.036**	-0.12 (-0.31;0.07)	.21	-1.40 (-3.43;0.63)	.17
Longitude ($_{hundredth}$°E)	-0.02 (-0.07;0.04)	.53	-0.10 (-0.27;0.08)	.28	-0.13 (-0.27;0.01)	.062	-2.40 (-3.91;-0.89)	**.0020**
Mar Grande (n=207)	**β (95%CI)**	**p**	**β (95%CI)**	**p**	**β (95%CI)**	**p**	**β (95%CI)**	**p**
Latitude ($_{hundredth}$°N)	0.03 (-0.04;0.11)	.39	0.15 (-0.40;0.71)	.59	0.19 (-0.38;0.76)	.51	2.38 (-0.59;5.36)	.12
Longitude ($_{hundredth}$°E)	0.09 (0.03;0.15)	**.0042**	-0.22 (-0.67;0.22)	.32	-0.23 (-0.64;0.18)	.26	2.93 (1.09;4.77)	**.0019**

Dioxins: sum of dioxins (WHO-PCDD/F-TEQ); DLPCBs: sum of dioxin-like PCBs (WHO-PCB-TEQ); dioxins+DLPCBs: sum of dioxins and dioxin-like PCBSs (WHO-PCDD/F-PCB-TEQ); NDLPCBs: sum of non-dioxin like PCBs: PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180 (ICES – 6); all pollutants: mean of the measured values, wet weight [16, 17].

except for I-IV comparison (p=0.18), differences in dioxins between all trimesters with higher values in trimester III (p_s<0.006); differences in Dioxins+DL-PCBs and DL-PCBs between all trimesters with higher values in trimester III (p_s<0.01); lower values of NDL-PCBs in trimesters I, II and IV compared to III (p_s<0.02).

Spatial distribution of the pollutants values within the three basins was shown in the left side of *Figure 1*. Results of multiple linear median regression for each basin and pollutant (values) were reported in *Table 3*. In Mar Piccolo 1st Inlet, all pollutants showed significant differences as regards to latitude, with an increase moving North (p_s<0.05). In Mar Piccolo 2nd Inlet, dioxins+DL-PCBs showed significant differences as regards to latitude, with a decrease moving North (p=0.036), while NDL-PCBs showed significant differences as regards to longitude, with a decrease moving East (p=0.0020). In Mar Grande both dioxins and NDL-PCBs showed significant differences as regards to longitude, with an increase moving East (p_s<0.005).

Results of Spearman rank correlation ρ for each basin and combination of pollutant (values) were reported in *Table 4*. All pairwise combinations in all basins showed significant correlation (p_s<0.0001). Dioxins+DL-PCBs

Table 4
Spearman rank correlation ρ for each basin and mussel pollutant (values)

2012-18		Mar Piccolo 1st Inlet (n=208)		Mar Piccolo 2nd Inlet (n=207)		Mar Grande (n=207)	
Poll. 1	**Poll.2**	**ρ**	**p**	**ρ**	**p**	**ρ**	**p**
Dioxins (pg/g)	Dioxins+DLPCBs (pg/g)	0.72	**<.0001**	0.82	**<.0001**	0.50	**<.0001**
Dioxins (pg/g)	DLPCBs (pg/g)	0.61	**<.0001**	0.67	**<.0001**	0.35	**<.0001**
Dioxins (pg/g)	NDLPCBs (ng/g)	0.74	**<.0001**	0.65	**<.0001**	0.59	**<.0001**
Dioxins+DLPCBs (pg/g)	DLPCBs (pg/g)	0.98	**<.0001**	0.97	**<.0001**	0.98	**<.0001**
Dioxins+DLPCBs (pg/g)	NDLPCBs (ng/g)	0.83	**<.0001**	0.82	**<.0001**	0.75	**<.0001**
DLPCBs (pg/g)	NDLPCBs (ng/g)	0.80	**<.0001**	0.82	**<.0001**	0.70	**<.0001**

Dioxins: sum of dioxins (WHO-PCDD/F-TEQ); DLPCBs: sum of dioxin-like PCBs (WHO-PCB-TEQ); dioxins+DLPCBs: sum of dioxins and dioxin-like PCBSs (WHO-PCDD/F-PCB-TEQ); NDLPCBs: sum of non-dioxin like PCBs: PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180 (ICES – 6); all pollutants: mean of the measured values, wet weight [16, 17].

seemed to be more correlated to DL-PCBs (0.98>ρ>0.97) than to dioxins (0.82>ρ>0.50), in particular in Mar Grande (ρ=0.98 vs ρ=0.50). The lowest correlation was found between dioxins and DL-PCBs in Mar Grande (ρ=0.35).

Temporal trends of the pollutants values over the observed years for each basin were shown in the left side of *Figure 2*.

Spatial distribution of the pollutants values above the EU maximum level between the three basins was shown in the right side of *Figure 1*. Overall pollutants values above EU maximum level frequency distribution between basins (p from Fisher-Freeman-Halton exact test) were:

- 4 (1.9%) in Mar Piccolo 1st Inlet and 0 (0.0%) in Mar Piccolo 2nd Inlet and Mar Grande for Dioxins>3.5 pg WHO-TEQ/g ww (p=0.036);
- 73 (35.1%) in Mar Piccolo 1st Inlet, 3 (1.4%) in Mar Piccolo 2nd Inlet and 0 (0.0%) in Mar Grande for dioxins+DL-PCBs>6.5 pg WHO-TEQ/g ww (p<0.0001);
- 38 (18.3%) in Mar Piccolo 1st Inlet, 2 (1.0%) in Mar Piccolo 2nd Inlet and 0 (0.0%) in Mar Grande for NDL-PCBs>75 ng/g ww (p<0.0001).

Pairwise Fisher exact test showed higher frequencies in dioxins+DL-PCBs>6.5 pg WHO-TEQ/g ww and NDL-PCBs>75 ng/g ww in Mar Piccolo 1st Inlet compared both to Mar Piccolo 2nd Inlet and Mar Grande for all pollutants (p_s<0.0001).

Mar Piccolo 1st Inlet pollutants values above EU maximum level frequency distribution between trimesters (p from Fisher-Freeman-Halton exact test) were:

- 0 (0.0%) in trimesters I and II, 2 (4.8%) in III and 2 (4.2%) in IV for Dioxins>3.5 pg WHO-TEQ/g ww (p=0.077);
- 2 (3.6%) in trimester I, 33 (53.2%) in II, 33 (78.6%) in III and 5 (10.4%) in IV for dioxins+DL-PCBs>6.5 pg WHO-TEQ/g ww (p<0.0001);
- 4 (7.1%) in trimester I, 16 (25.8%) in II, 15 (35.7%) in III and 3 (6.2%) in IV for NDL-PCBs>75 ng/g ww (p=0.0001).

Pairwise Fisher exact test showed: except for I-IV comparison (p=0.24), differences between all trimesters in dioxins+DL-PCBs>6.5 pg WHO-TEQ/g ww with higher frequency in III (p_s<0.02); lower frequencies of NDL-PCBs>75 ng/g ww in both trimesters I and IV compared both to II and III (p_s<0.02).

Spatial distribution of the pollutants values above the EU maximum level within the three basins was shown in the right side of *Figure 1*. Results of multiple binary logistic regression for Mar Piccolo 1st Inlet and NDL-PCBs>75 ng/g ww showed significant differences as regards to latitude, with a frequency increase moving North (OR 6.43, 95%CI 2.66;17.00, p=0.0001), while no differences were found as regard to longitude. No significant differences were found for DL-PCBs>6.5 pg WHO-TEQ/g ww (p>0.05).

All pairwise combinations of pollutant (values above EU maximum level) in Mar Piccolo 1st Inlet showed significant Pearson correlation ϕ (p_s<0.006), with low correlation of dioxins>3.5 pg WHO-TEQ/g ww with dioxins+DL-PCBs>6.5 pg WHO-TEQ/g ww (ϕ=0.19) and with NDL-PCBs>75 ng/g ww (ϕ=0.21) and higher correlation of dioxins+DL-PCBs>6.5 pg WHO-TEQ/g ww with NDL-PCBs>75 ng/g ww (ϕ=0.54).

Temporal trends of the pollutants values above the EU maximum level over the observed years for each basin were shown in the right side of *Figure 2*.

DISCUSSION

As mussel farming historically represents an important cultural and economic heritage for the citizens of Taranto, the Department of Prevention of the Local Health Authority have long been engaged on the dual front of protecting consumers and safeguarding primary production.

The results of our study confirmed higher levels of dioxins and PCBs concentrations as well as values exceeding the EU maximum levels mainly in Mar Piccolo 1st Inlet. This finding supports the validity of the Regional Ordinance 188/2016 and the consequent Public Health measures carried out by the Department of Prevention of the Local Health Authority of Taranto. The prohibition of exploiting the Mar Piccolo 1st Inlet for mussel farming, except for juvenile mussels handling by February 28, intercepts almost all the exceedances observed with respect to the EU limits for dioxins and PCBs and plays a pivotal role in ensuring the healthiness of the product placed on the market.

Higher levels of dioxins and PCBs concentrations as

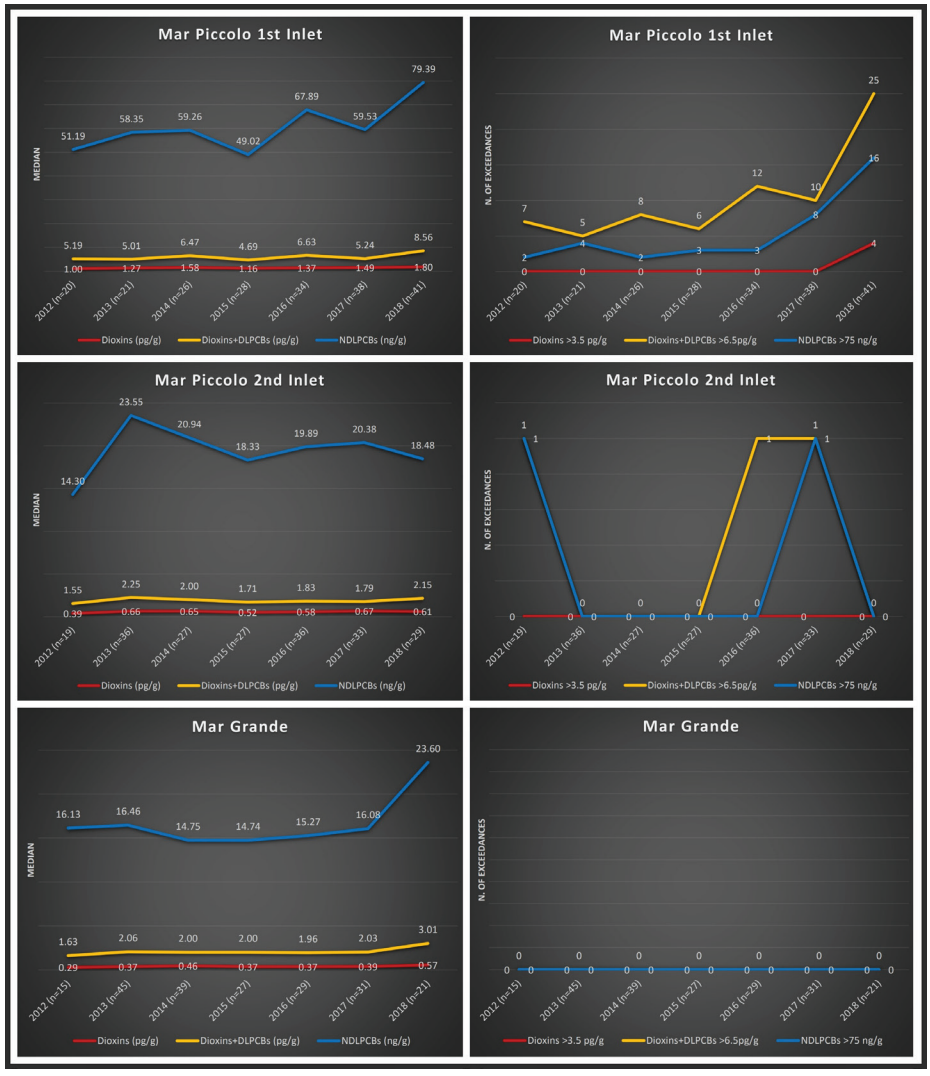

Figure 2
Temporal trends of mussel pollutants (values and values above EU maximum level) over the observed years.
Dioxins: sum of dioxins (WHO-PCDD/F-TEQ); DLPCBs: sum of dioxin-like PCBs (WHO-PCB-TEQ); dioxins+DLPCBs: sum of dioxins and dioxin-like PCBSs (WHO-PCDD/F-PCB-TEQ); NDLPCBs: sum of non-dioxin like PCBs: PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180 (ICES – 6); pollutants on the left: mean of the measured values, wet weight; pollutants on the right: mean of the measured values minus the expanded uncertainty of the mean, wet weight [16, 17].

well as values exceeding the EU maximum levels in Mar Piccolo 1st Inlet can be explained by the proximity of industrial settlements, which account for known potential sources of PCDD/Fs and PCBs, e.g. contaminating groundwater and freshwater in the northern area of the basin [14]. This is in line with the higher measured PCBs concentrations in marine sediments from Mar Piccolo 1st Inlet (Range 54 – 1684 µg/kg dw) compared to 2nd Inlet (Range 2 – 181 µg/kg dw) [13]. It is therefore not surprising that the mussels produced in Mar Piccolo 2nd Inlet and Mar Grande basins exhibited lower dioxins and PCBs concentrations which were basically always below the EU maximum levels.

In order to effectively deal with mussels contamination in Taranto, another key issue to consider is certainly the marked seasonality of measured dioxins and PCBs concentrations. As a matter of fact, summer is undoubtedly the most critical period, in accordance with a dependence of mussels filtration rate from temperature, as yet reported in literature [18-20]. The influence of temperature on filtration activity seems to be higher between 5°-15°C and 25°-30°C, lower from 15° to 25°C, while at 5° and 30°C, filtration drops to very low values [18]. The linear increase of filtration rate with temperature in mussels can be explained by increased biological activity as well as by decreased water viscosity [19, 20]. In Mar Piccolo, the scarce hydrodynamism and the low water exchange with Mar Grande determine, mainly in summer, a high water stratification and a significant increase in the average water temperature compared to the open sea [13].

Besides, the observed seasonal concentrations pattern could be linked to cyclical variations of mussels physiological state and lipid content during the year re-

lated to their reproductive cycle [14]. Infact, in the later stages of gametogenesis (March-May), the increase in mussels lipid content may lead to a greater risk of bioaccumulation due to the strong lipophilic properties of dioxins and PCBs [1, 5].

Our results showed also a differential fluctuation of dioxins and PCBs concentrations during the year, with higher dioxins concentrations during the 3rd trimester and higher PCBs concentrations during both 2nd and 3rd trimester. To explain this different temporal pattern, we could hypothesize a difference between dioxins and PCBs toxicokinetics in mussels that account for a delay in dioxins concentrations increase compared to PCBs' and, maybe, for higher PCBs measured concentrations. In fact, is of the utmost importance to underline that the majority of observed EU limits exceedances (96.7%) referred to PCBs rather than dioxins, suggesting that PCBs constitute the most abundant contaminant in marine sediments of Taranto [13]. This finding is of particular Public Health relevance considering that exposure to PCBs is associated with melanoma (sufficient evidence), Non-Hodgkin lymphoma and breast cancer (limited evidence) [21].

Moreover, early rise of PCBs concentrations starting from the 2nd trimester corroborates the effectiveness of the Regional Ordinance 532/2018, which anticipates the temporal limit for juvenile mussels handling to February 28, a choice that has also been considered in relation to the Southern Italy climate pattern of last years, characterized by a progressive increase in temperatures linked to climate change [22].

On the other hand, the possibility of moving juvenile mussels (<5 cm) from Mar Piccolo 1st Inlet to the other basins by February 28 is confirmed to be an option that guarantee the healthiness of the product, in light of the fact that 100% of the detected exceedances in Mar Piccolo 1st Inlet during the I trimester refers to commercial size mussels (≥5cm).

An interesting finding of this study is the linear relationship between some pollutants and geographical coordinates. In Mar Piccolo 1st Inlet, there was a linear increase of dioxins and PCBs moving North, in accordance with the marine sediments resuspension near the northern coast. In Mar Piccolo 2nd Inlet, we found a linear increase of some pollutants moving South or West, i.e. moving towards the Mar Piccolo 1st Inlet entrance channel. In Mar Grande, there was a linear increase of some pollutants moving East, in accordance with the marine sediments resuspension near the eastern coast. Marine sediments resuspension near the coast could be explained by several factors, e.g. low water depths, boats passage, water agitation by groundwater and freshwater flowing, human activities and, near the North-Western coast of Mar Piccolo 1st Inlet, a significant water outflow due to the presence of water-scooping machines that supply seawater to the steel plant cooling system. Finally, the correlation analysis showed stronger correlation between most of the pollutants in Mar Piccolo, leading to the hypothesis of related sources of contamination, while in Mar Grande the weaker correlation between dioxins and PCBs may suggest different unrelated pollution sources.

The possibility to identify a relationship between the levels of mussels contamination and the marine sediments resuspension stimulates an important reflection of public health. As a matter of fact, regardless the public health measures carried out by the Regional Ordinances as well as by the Local Health Authority have effectively remedied an emergency situation, nevertheless they cannot be considered definitive solutions, as the only way to deal with the problem of persistent organic pollutants contamination in Taranto basins is the remediation of contaminated marine sediments. In particular, in Mar Piccolo 1st Inlet, where we may suppose a common source sediments contamination (as suggested by the strong correlation between the pollutants), an environmental remediation plan would meet the needs of safeguarding the health of consumers as well as of ensuring the long-term survival of mussel farming.

In fact, the prohibition of exploiting the water body of the Mar Piccolo 1st Inlet for the mussel farming, on one hand forced mussel farmers to use almost exclusively the area of Mar Piccolo 2nd Inlet, leading to a significant overcrowding of installations, to the death of mussels and to the overall loss of most of the product legally raised in recent years, on the other hand to an increase of illegal production of unsafe mussels in Mar Piccolo 1st Inlet, which raises a serious public health concern.

As a partial solution, to deal with the contamination of the sediments in Mar Piccolo 1st Inlet as well as with the water warming and overcrowding in the Mar Piccolo 2nd Inlet, the further implementation of mussel farming in Mar Grande could favor the exploitation of many currently unused areas. However, given the peculiar environmental, geographical and cultural context of Taranto, the health protection cannot be separated from a rigorous policy action to reduce pollutant emissions, as well as from the environment restoration, which is also a key ethical issue. Moreover, the EU WFD (Water Framework Directive) requires Member States to achieve a good chemical status for all waterbodies in Europe as regard to dioxins and DL-PCBs, which are classified as priority substances in the field of water policy [23]. Anyhow, the sediment remediation plan should be carefully and appropriately applied in order to preserve the ecosystem of the area.

Finally, analyzing the problem from an historical perspective, the temporal trends of the pollutants over the observed years showed a concentrations increase in 2018 in Mar Piccolo 1st Inlet and Mar Grande. We do not know the reasons for this finding, but we can make some hypotheses: rising water temperatures, increase in human activities in the basins causing sediment resuspension, or, in Mar Piccolo 1st Inlet, overcrowding of installations leading to the death of the product and to the consequent release of accumulated pollutants in the water near the other filtering mussels.

In conclusion, a structured environmental plan for remediation of contaminated marine sediments, alongside with the reduction of pollutant emissions, appears to be the only effective, efficient and ethical long-term solution to protect the health of consumers, preserve the local production chain and restore the marine environment.

Conflict of interest statement
None.

Received on 23 July 2020.
Accepted on 6 October 2020.

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