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Baseline

Occurrence and distribution of polycyclic aromatic hydrocarbons in mussels from the gulf of Naples, Tyrrhenian Sea, Italy

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ABSTRACT

To assess the potential impact of the industrial activity on food safety and risk for consumers, the aim of the study was to evaluate the levels of 14 polycyclic aromatic hydrocarbons (PAH) in 69 samples of wild and farm *Mytilus galloprovincialis*, collected in sites of coast of Gulf of Naples, Tyrrhenian Sea.
All hydrocarbons were found in samples. Higher levels of pyrolytic PAHs were in wild than in farm mussels. Benzo(*a*)pyrene exceeded the Regulation (EC) n.835/11 levels of 1 μg/kg in 15 samples (71.42%) of wild and 25 samples (65.79%) of farm mussels. System of sum of 4 hydrocarbons exceeded the law level in 15 samples (71.42%) of wild and 21 samples (55.26%) of farm mussels. Wild mussel levels showed a potential impact of pyrolytic sources of PAH on food safety. Occurrence of carcinogenic PAHs should be a cause for concern, in areas where the mussels are being farmed for human consumption.

The environmental quality of the marine ecosystems in the Gulf of Naples, marginal basin of the southeastern Tyrrhenian Sea, Italy, is directly influenced by human activities. Waters of the Gulf present hydrographic and biological properties reflecting anthropic stress (Ribera d'Alcalà et al., 1989; Zingone et al., 1990, 2006; Uttieri et al., 2011). Farming of mussels for human consumption is a common practice in this area (Tornero and Ribera d'Alcalà, 2014).

Polycyclic aromatic hydrocarbons (PAHs) are hazardous environmental chemicals with carcinogenic and mutagenic properties (Neff, 1979; Piccardo et al., 2001). As pollutants PAHs enter in the marine environment from a variety of sources: petrogenic (low molecular weight) compounds as the result of spillage of diesel oil and/or fuel oil, and pyrolitic (medium and high molecular weight) PAHs produced by the incomplete combustion of organic matter (Srogi, 2007; Tornero and Ribera d'Alcalà, 2014). Being filter-feeding, wild mussels have been used as sentinel organism for monitoring the uptake of hydrophobic contaminants, including PAHs in costal environment (Livingstone et al., 1992; Storelli and Marcotrigiano, 2001; Soriano et al., 2007). On the other hand mussels for human consumption represent an important source of human exposure to PAHs. Commission Regulation (EC) n.835/, 2011 fixed a maximum level of 6 μg/kg for Benzo(*a*)pyrene (BaP) as marker, and of 35 μg/kg for the system of sum of 4 hydrocarbons as marker, that is Benzo(*a*)pyrene, Chrisene, Benzo(*a*)nthracene, and Benzo(*b*)Fluoranthene (∑PAH4) in bivalve mollusks (EFSA, 2008).

The aim of the study was to investigate the PAH contamination of wild and farmed mussels collected in the Gulf of Naples in order to assess the potential impact of industrial activity on the food safety and risk for consumers.

Mediterranean mussels (*Mytilus galloprovincialis*) were collected by trawling to a depth of 30–60 m in marine areas of Bays of Pozzuoli and Naples, located on the northeastern coast of the Gulf. A total number of 69 samples were analyzed: 48 samples were collected from 16 breeding farms situated in classified harvesting sites, while 21 samples of wild mussels from no classified marine area located at 2.7 nautical miles from classified harvesting sites (Fig. 1).

Levels of total PAHs, PAHs markers, and concentrations of carcinogenic PAHs were studied. From each site a pool of 30 individuals was collected and frozen at −20 °C until processing. Three replicate samples were carried out. About 1.5 g of homogenized tissue was saponified by 10 mL of 1 M KOH in an ethanol solution for 3 h at 80 °C in a water bath. Then 10 mL of water and 20 mL of cyclohexane were added, the samples were mixed by an orbital agitator for 5 min and stabled for 10 min The hexanic phase was recovered and the polar mixture was rinsed twice with two aliquots of cyclohexane. The extracts were filtered through filter paper, filled with sodium sulfate anhydrous and run-on a column filled with Florisil. The eluates were dried under a flow of air and dissolved in 1 mL of acetonitrile before the analysis (Dafflon et al., 1995).

Quantitative analysis of PAHs was carried out using HPLC equipped with UV detector. PAHs were separated at ambient temperature using

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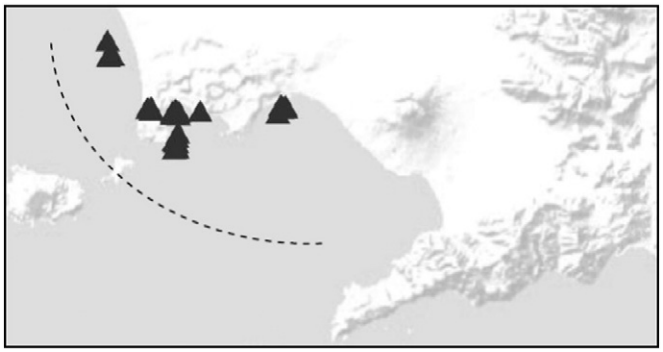

Fig. 1. Location of sampling sites along the northeastern coast of Gulf of Naples, Tyrrhenian Sea. Legend: ▲ = farm mussel site, ---- = wild mussel sites.

a C18 Envirosep-PP column 5 μm (125 mm × 4.6 mm) (Phenomenex) and a gradient elution program with a flow rate of 0.6 mL/min. At the beginning of the mobile phase, water was 60% and acetonitrile was 40% in HPLC. Acetonitrile was then gradually changed to 100% in 20 min, held at 100% for 27 min, and then decreased to the initial phase in 30 min. The different UV wavelengths for each compound are shown in Table 1.

Analytes were identified on the basis of retention time. Quantification of PAHs was performed by using an external standard method. PAH-mix9 standard (Dr Ehrenstorfer, Reference Materials, Augsburg, Germany, 100 ng/μL in acetonitrile), containing 14 PAHs frequently occurring in food (Table 2) was used for the preparation of working standard solutions (5, 10, 25, 50, 100 ng/mL in acetonitrile). QA/QC was ensured by the use of matrix spikes and spiked duplicates. Accuracy was determined by analyzing the reference materials. Analyte recoveries were determined by using unpolluted mussel tissue spiked with solutions containing 25, 50, 100 ng/mL of the PAH standard. Detection limit (LOD), as signal to-noise 3:1 and quantification limit (LOQ), as signal-to noise 6:1, were calculated. External standard multipoint calibration technique was used to determine the linear response interval of the detector, and regression coefficients were 0.9852.

Statistical data of analysis was performed with package SPSS 15.0 (SPSS Inc., Chicago). One-way ANOVA test were applied to determine significant differences ($P < 0.05$) among groups (according to origin of samples, molecular weight, marker levels, and carcinogenic potency of PAHs).

In samples of wild and farm mussel collected in Bays of Napoli and Pozzuoli all researched hydrocarbons were found. Total PHA levels ranged (min–max values) from 4.47–905.66 μg/kg in wild, and 0.71–1314.45 μg/kg in farm mussels (Fig. 2). In wild mussels Chr (max 163.07 μg/kg) and BkF (max 178.46 μg/kg) characterized the PAH profile, while in farm mussels Chr (max 214.74 μg/kg) and Py (max 218.09 μg/kg) showed the highest concentrations. Wild mussels showed higher levels of pyrolytic compounds than farm mussels, and no significant differences were observed regarding medium and low molecular weight PAHs in two groups of mussels (Fig. 3).

Level of PAH markers in wild and farm mussels are reported in Fig.4. BaP and ∑PAH4 showed higher level in wild than farm mussels ($P < 0.05$). In particular BaP exceeded the regulatory level in n.15 samples (71.42%) of wild and n.25 (65.79%) of farm mussels (min–max values 0.13–78.50 μg/kg, and 0.03–64.20 μg/kg, respectively). Data on ∑PAH4 showed that levels exceeded the limit in n.15 samples (71.42%) of wild and n.21 samples (55.26%) of farm mussels (min–max values 0.52–285.24 μg/kg and 0.36–281.87 μg/kg, respectively).

Table 1
Program of excitation and emission wavelengths during PAH analysis.

Time (min)	Excitation (nm)	Emission (nm)
0	252	402
7.6	238	398
8.5	268	398
11.5	300	466
16.0	300	466

Table 2
Low, medium and high molecular weight investigated PAHs.

Petrogenic PAHs Low molecular weight	Pyrolitic PAHs Medium molecular weight	Pyrolitic PAHs High molecular weight
Acenaphtene (Ap)	Fluoranthene (F)	Benz(*a*)anthracene (BaA)
Fluorene (Fl)	Pyrene (Py)	Chrysene (Chr)
Phenanthrene (Phen)		Benzo(*b*)fluoranthene (BbF)
Anthracene (A)		Benzo(*k*)fluoranthene (BkF)
		Benzo(*a*)pyrene (BaP)
		Dibenz(*a*,*h*)anthracene (DBahA)
		Benzo(*g*,*h*,*i*)perylene (BghiP)
		Indeno(1,2,3-*cd*)pyrene (Icdpy)

International Agency for Research of Cancer (IARC) has determined that BaP is a compound of *Group* 1—*carcinogenic to humans*, whereas DBahA has been classified as *Group* 2A—*possible human carcinogenic*, and BaA, Chr, BbF, BkF, IcdPy have been classified as B2—*probable human carcinogenic* (IARC, 2011). Results showed an occurrence of carcinogenic compounds in mussels collected in the Gulf of Naples, and significant ($P < 0.05$) higher levels of *carcinogenic*, *possible* and *probable carcinogenic* hydrocarbons in wild mussels (Fig. 5).

Gulf of Naples is subject to PAH pollution by the proximity to major coastal settlements and insufficient rate of diffusion due to its semi-enclosed nature. In analyzed wild and farm mussels the total PAH levels seem to confirm an increase in PAH inputs with time. Possible emission sources for PAHs are reported (Meriç et al., 2005). Industries are mostly concentrated in the northern half of the regional territory, especially next to main cities, and devoted to vegetable preserving processes, textile-apparel, clothes production and leather tannery. Inadequate purification systems in the tannery and vegetable preserving activities can lead to significant pollution levels in stream waters and sediments, resulting in a number of health effects on crops, aquatic and terrestrial biota, and humans (Meriç et al., 2005). Bagnoli industrial area in the Bay of Pozzuoli was dominated by the presence of a steel plant (ILVA), that was in operation from 1905 until 1990 (Romano et al., 2004). Assessment of hydrocarbon pollution area of the industrial site of Bagnoli exhibits elevated PAH concentrations, both in surface and bottom sediments (Tornero and Ribera d'Alcalà, 2014). In this area the highest concentrations were detected in the samples of mussels closer to the industrial plant (Romano et al., 2004). Naples area has been described as a hot spot of PAH pollution in the western basin of the Mediterranean Sea (Andral et al., 2011; Tornero and Ribera d'Alcalà, 2014). Generally far from strongly urbanized and industrial areas mussels, sampled from the northern coast of Gulf of Naples, presented relatively low PAH levels (SiDiMar, 2005; Perugini et al., 2007a; Tornero and Ribera d'Alcalà, 2014) (Fig. 6).

In our study the highest concentrations of the total PAHs in wild mussels collected in the Gulf of Naples might be related to nearby contamination sources, if compared to those of farm mussels, sampled away from point sources. It is possible that, away from pollution sources, there is a threshold below which the farm mussels balance the uptake and depuration of PAHs, thus maintaining a relatively constant concentration, according to literature (Baumard et al., 1998; Baumard et al., 1999; Guinan et al., 2001). In addition the observed variations in the sum of PAHs in wild and farmed mussels could be caused by different physiological conditions in the mussel populations, related to the different stages in their lifecycle at the time of sampling, as Jacob et al. (1997), Baumard et al. (1999) and Law et al. (1999) reported. PAH levels might be related also to hydrodynamics in the Gulf of Naples (Perugini et al., 2007a), and seasonal differences of biotic and abiotic