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PAH (Polycyclic Aromatic Hydrocarbon) bioaccumulation and PAHs/shell weight index in *Ruditapes philippinarum* (Adams & Reeve, 1850) from the Vallona lagoon (northern Adriatic Sea, NE Italy)

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ABSTRACT

The Vallona lagoon is a transitional area located in the Po River delta (NE, ITALY) traditionally exploited for Manila clam (*Ruditapes philippinarum*) farming. During 2007–2008, a pipeline was buried in the middle of the lagoon to connect an off-shore structure to facilities on land. PAH levels were monitored in Manila clams and sediments before, during and after the pipeline construction to assess the impact of the activities through the pattern of distribution of the PAH compounds.

PAH bioaccumulation in clams displayed seasonal fluctuations with higher levels in autumnal and wintry surveys than in spring-summer. Principal component analysis applied to PAHs in clams highlighted a petrogenic input during *ante operam* period and a pyrolytic origin during the burying activities. On the contrary, sediment PAH concentrations resulted quite similar both among sites and periods. Biota-Sediment-Accumulation-Factor values also confirmed that sediments were not the major source of PAH pollution for clams in this study.

The welfare of clams was examined through two physiological indices (condition index and survival in air) to check the effects of the activities on a commercial resource. Both physiological indices exhibited seasonal variations connected to natural endogenous and exogenous factors; however survival in air was the most sensitive index in highlighting the effects of the pipeline burying activities.

Finally, to ensure that PAH bioavailability assessment was not affected by seasonal variation of soft tissues of molluscs, PAHs/shell weight index was applied. Higher levels of this index were observed before and during the burying activities, whilst, after that, values significantly lowered. Moreover, the normalization enabled us to highlight the PAH uptake from clams in some particular periods and to compare different populations in a long-term biomonitoring program with data obtained from different periods of the year.

1. Introduction

Among persistent organic pollutants (POPs), polycyclic aromatic hydrocarbons (PAHs) have been considered of critical concern due to their potential carcinogenicity, mutagenicity and teratogenicity to aquatic organisms and human beings (Kennish, 1997). PAHs are present in most coastal areas, accumulating in sediments and organisms, especially those which have low capability to eliminate them, such as bivalves (Meador et al., 1995). The anthropogenic sources of PAHs in marine and estuarine environments could be either petrogenic or pyrolytic, and the pattern of distribution of these compounds can be used to get information about their origin, as they impart a unique PAH signature in the marine and transitional environments (Oros and Ross, 2005 and authors therein). Indeed, low molecular weight (LMW-)PAHs with up to four-rings are considered from petrogenic input, such as oil spills, whilst high molecular weight (HMW-)PAHs with more than four-rings are from pyrolytic input, including thermal combustion processes (Neff, 1979; Simoneit, 1984). Introduction into marine and transitional environments of combustion derived PAHs, coming from vehicular emissions or biomass burning, can be through air-water interface, dry deposition of airborne particulate matter or wet deposition by rainfall. Bivalves can accumulate them indirectly through ingestion of sediments and assimilated through the digestive tract. On the contrary, petroleum PAHs, which can enter through urban runoff, stormwater discharge or oil spill, are more biodegradable than combustion derived PAHs and once present in the water column they can be easily accumulated by

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bivalves through their gills (Simoneit, 1984; Baumard et al., 1998; Oros and Ross, 2005).

Ruditapes philippinarum is a soft-bottom dweller bivalve commonly used in biomonitoring programs, especially in bioaccumulation assessment, owing to its high tolerance to toxic compounds (Moschino et al., 2012). As bivalves, Manila clams have indeed quite a low enzyme activity to metabolize POPs, such as PAHs, and their tissue concentrations can be a good indicator of exposure level of environmental contamination from these compounds (Meador et al., 1995; Otchere, 2005; Lee et al., 2014). However bioavailability and accumulation of contaminants in the soft tissue of molluscs could be affected by abiotic factors, such as food availability, pH and temperature, and also by biotic factors, such as seasonal changes of flesh weight in molluscs. To overcome this problem, Fischer (1983) proposed to use the metal/shell weight index to assess the heavy metal bioavailability which consists of normalization achieved multiplying metal concentrations by the condition index. The same index was then used also for PAHs, showing to be a good tool to obtain results not affected by seasonal changes in flesh weight and by stress conditions of clams *R. philippinarum* used in active biomonitoring (Boscolo et al., 2007).

The Vallona lagoon is a transitional area of about 1150 ha located in the Po River delta, the largest and most important Italian watercourse (Vincenzi et al., 2014). This lagoon has been traditionally exploited for aquaculture, especially for shellfish, and about one-fifth of the total (about 200 millions €) northern Adriatic Sea production of clam (*R. philippinarum*) farming comes from the Vallona lagoon (Turolla, 2008; Abbiati et al., 2010). During 2007–2008, a pipeline was buried in the middle of the lagoon to connect an off-shore structure to facilities on land. In view of this, an environmental monitoring program was scheduled from 2005 to 2014, which consisted of three phases: (i) before, (ii) during and (iii) after the construction of the structures. One of the aims of the monitoring was to investigate the disturbances on the lagoon clam population, including surveys to monitor the welfare of bivalves and their bioaccumulation of contaminants.

In this context, the aim of this study was to monitor PAH levels in Manila clams collected before, during and after burying the pipeline in the Vallona lagoon in order to assess the impact of the activities through the pattern of distribution of the PAH compounds. The welfare of clams was also examined through physiological measurements in order to check the effects of the activities on a commercial resource. Beyond this, the present results were also discussed in order to assess if the general environmental conditions of the area and other PAHs input could affect the lagoon clam population. Therefore, sediment samples were collected to investigate the bioavailability of sediment-associated PAHs and, in order to ensure that PAH bioavailability assessment was not affected by seasonal variation of soft tissues of molluscs, PAHs/shell weight index was applied.

2. Materials and methods

2.1. Sample collection and preparation

The survey was conducted in the Vallona lagoon, NE Italy (Fig. 1). Manila clams (*R. philippinarum*) were gathered by manual rake at four sites: L016 and L017 are located farther from the pipeline, whilst L022 and L023 are closer to the pipeline. Sediments were collected by box corer at sites L016 and L017.

Clams were sampled on November 2005, February, April and July 2006 (*ante operam* phase), November 2006, February 2007, June and November 2008 (*in opera* phase), November 2010, June and November 2011, June and November 2012, May and November 2013, and June 2014 (*post operam* phase). Sediments were sampled on November 2005 (*ante operam* phase), August 2008 (*in opera* phase), June 2011 and 2012, May 2013 and 2014 (*post operam* phase).

After collection, molluscs were transported immediately to the laboratory in cold and dark conditions. Then, for every sample, each clam was washed under running water to remove any surface deposits from the shell.

About thirty clams per sample were opened by cutting the posterior adductor muscle, rinsed with reagent water to eliminate any sediment residues, and the excess of water and body fluid was left to drip before collecting the soft tissues for chemical analyses. These soft tissues were then pooled (three pools of ten clams per sample), weighed, homogenized and maintained at −20 °C until analysis.

Eighty clams per sample were used for the biological indices.

Sediments, after collection, were thoroughly homogenized and immediately transferred into clean polyethylene jars and kept at −20 °C until analysis.

2.2. Determination of PAHs

2.2.1. Standards and reagents

A standard solution containing a mixture of 16 PAHs (Naphthalene, Acenaphthylene, Acenaphthene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Benzo[a]Anthracene, Chrysene, Benzo[b] Fluoranthene, Benzo[k]Fluoranthene, Benzo[a]Pyrene, Dibenzo [a,h]Anthracene, Benzo[g,h,i]Perylene and Indeno[1,2,3,c,d]Pyrene) was purchased from Ultra Scientific (USA); the standard solutions for 1-Fluoronaphthalene, 3-Fluorophenanthrene and 1-Fluoropyrene were purchased from Chiron AS (Norway). The analytical grade organic solvents (methanol, dichloromethane, n-hexane, acetonitrile, isopropanol), as well as anhydrous sodium sulphate (Na_2SO_4), silica gel and sodium hydroxide (NaOH) were purchased from different manufacturers over the years: J.T. Baker (USA), Carlo Erba Reagents S.r.l. (Italy), Sigma-Aldrich Co. LLC (USA), Biosolve Chimie (France).

2.2.2. Analytical procedure

The biota samples were freeze-dried in order to obtain sample stability and enhance solvent extraction efficiency. The freeze-dried samples were finely sliced and homogenized by means of an electric mill fitted with a cutting blade (IKA-Werke GmbH & Co. KG, Germany). The dry, homogenized sample was then subjected to pressurized fluid extraction (PLE) using a Dionex (now Thermo Scientific Inc.) ASE 200 instrument. A 1.0 g aliquot of biota sample was extracted using a 40%n-hexane–60%dichloromethane mixture. In-cell clean up was conducted by inserting on the bottom of the extraction cell an 8 g layer of silica gel deactivated with 15% water (m/m). The resulting extract was then concentrated to 3–5 mL and underwent a second clean up step consisting of a cold saponification by shaking with a methanolic solution of NaOH. The PAHs were back-extracted with an n-hexane-dichloromethane (80:20 v/v) mixture which was passed on anhydrous Na_2SO_4 and then concentrated. A 5 mL isopropanol aliquot was added as a keeper and the extract was concentrated to a final volume of 1–1.3 mL (gravimetric determination). In order to minimize the loss of the most volatile analytes, the solvent concentration steps were performed employing particularly gentle conditions: no heating was provided, no vacuum was applied and no nitrogen blowing was employed, relying on a spontaneous evaporation of the solvent by simple hood aspiration. These conditions enabled to fulfil the requirements for recovery (60–120%) also for the most volatile compounds. The extract was then filtered on 0.2 μm and analysed by HPLC or stored in a freezer.

The sediment samples were freeze-dried in order to obtain sample stability and enhance solvent extraction efficiency. The freeze-dried sediment samples were passed through a 2 mm stainless steel sieve and then finely homogenized by means of an electric mill fitted with a grinding beater (IKA-Werke GmbH & Co. KG, Germany). The dry, sieved and homogenized sample was then subjected to pressurized fluid extraction (PLE) using a Dionex (now Thermo Scientific Inc.) ASE 200 instrument. A 5.0 g aliquot of sediment sample was extracted using a 40%n-hexane–60%dichloromethane mixture. The resulting extract was