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Baseline

Dioxin-like compounds bioavailability and genotoxicity assessment in the Gulf of Follonica, Tuscany (Northern Tyrrhenian Sea)

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ABSTRACT

The Gulf of Follonica (Italy) is impacted by the chemical pollution from ancient mining activity and present industrial processes. This study was aimed to determine the bioavailability of dioxin-like compounds (DLCs) in coastal marine environment and to assess the genotoxic potential of waste waters entering the sea from an industrial canal. Moderately high levels of DCLs compounds (ΣPCDDs + PCDFs 2.18–29.00 pg/g dry wt) were detected in *Mytilus galloprovincialis* transplanted near the waste waters canal and their corresponding Toxic Equivalents (TEQs) calculated. *In situ* exposed mussels did not show any genotoxic effect (by Comet and Micronucleus assay). Otherwise, laboratory exposure to canal waters exhibited a reduced genomic template stability (by RAPD-PCR assay) but not DNA or chromosomal damage. Our data reveal the need to focus on the levels and distribution of DLCs in edible species from the study area considering their potential transfer to humans through the consumption of sea food.

The chemical contamination of coastal marine environments often results from the exploitation of local resources spanning over millennia. This is the case of the Gulf of Follonica (Tuscany, Italy) that has been under the influence of both ancient metallurgic (particularly in Etruscan-Roman times and Middle Ages) activity (Costagliola et al., 2008) and recent industry which used ores in their processes and were responsible for a remarkable elevation of metal levels in the coastal marine environment (Regoli and Orlando, 1994). These features attracted the interest of ecotoxicologists aimed to *in situ* study metal bioaccumulation, toxicity and detoxification in mussels (Regoli, 1992; Regoli and Orlando, 1994; Regoli and Principato, 1995). In face of the vast amount of data on metal pollution, information about the presence and bioavailability of Persistent Organic Pollutants (POPs) are very scarce.

The present study is aimed to investigate the bioavailability of Dioxin-Like Compounds (DLCs) in the coastal marine environment of the Gulf of Follonica interested by the waste water carried by Canale Solmine (artificial canal receiving waste water from local industries and municipal treatment plant) and to assess the genotoxic potential of canal water for the Mediterranean mussel (*Mytilus galloprovincialis*) both after transplantation and laboratory exposure.

Dioxin-like compounds were selected being produced during industrial incinerating/combustion processes and responsible for toxic effects in humans and wildlife species. These compounds share the same exposure route (through the diet), mechanism of action and adverse additive effects, including disorders of the immune, reproductive, and endocrine systems (White and Birnbaum, 2009). The health risk associated with their bioaccumulation, expressed by the 2,3,7,8-TCDD toxic equivalents (TEQs) (Safe 1990; Van den Berg et al., 2006), was were also calculated.

Genotoxicity evaluation included DNA damage (assessed by the Comet assay), chromosomal alterations (assessed by Cytome-Micronucleus assay) and Genome Template Stability (GTS) reduction (assessed by RAPD-PCR).

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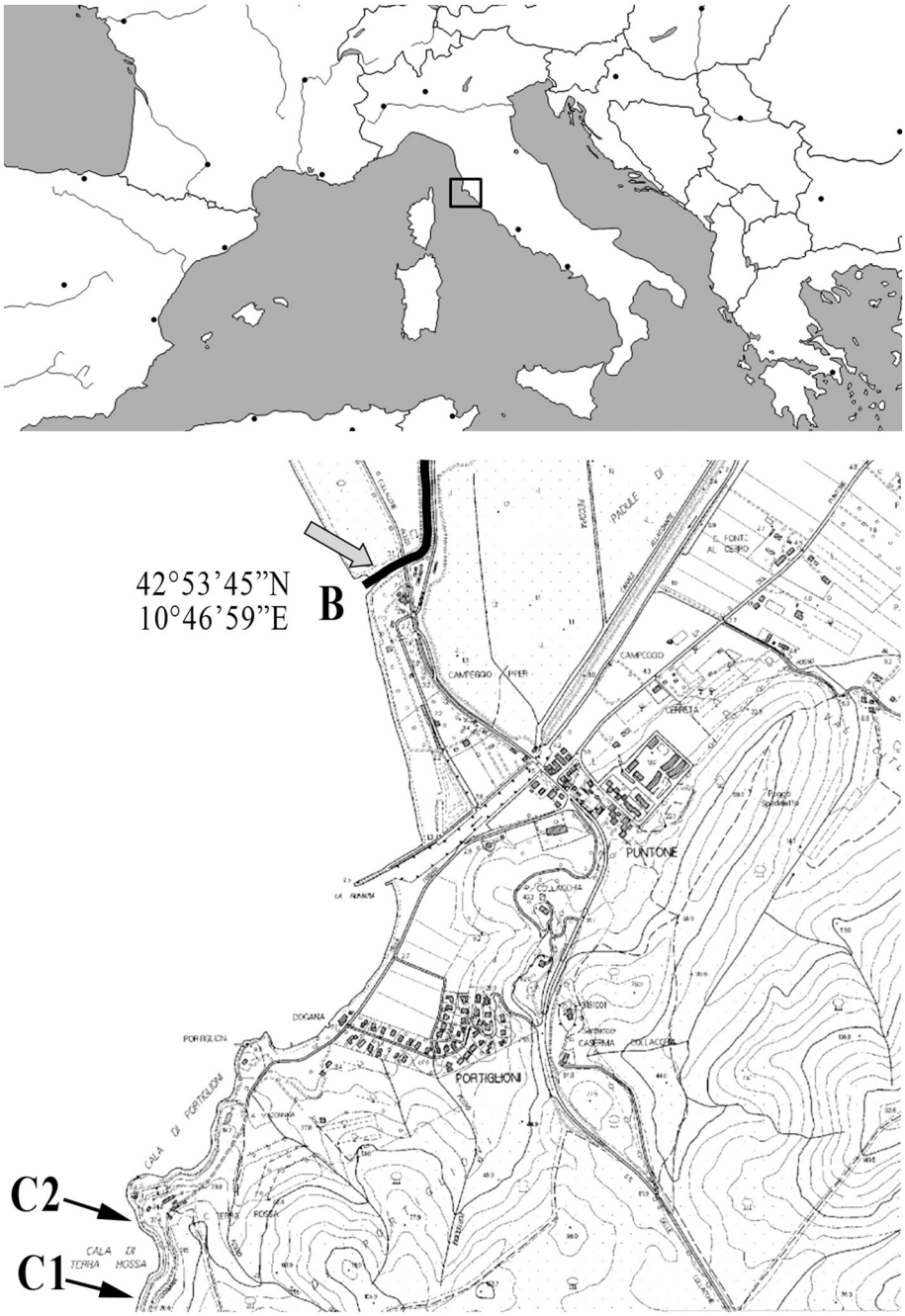

Fig. 1. Study area and sampling sites. Grey arrow: Canale Solmine, B: buoy ste, C1: control site 1, C2: control site 2. source: Regione Toscana - SITA Cartoteca (available at http://www502.regione.toscana.it/geoscopio/cartoteca.html).

For *in situ* investigation, mussels (obtained from Arborea mussel farm, Sardinia Island, Italy) were translocated about 100 m offshore the outlet of the Canale Solmine, attached to a buoy (site B) and in two putative reference sites (C_1, C_2) located about 5 km SW of the study site (Fig. 1). At the end of the exposure period (4 and 14 days) mussels were sampled, wrapped in a wet tissue and transported to the laboratory in a cold box for chemical and genotoxicity analysis. For laboratory exposure, mussels from the farm were maintained 48 h before the experiment in a flow-through circulating 100-L aquaria at 16 °C, constant photoperiod (12:12 light:dark). Successively, specimens (n = 45) were transferred into glass 10 l aquaria (n = 15 specimens each) and exposed to the water collected 50 m upstream the mouth of Canale Solmine (N. 2 aquaria). Negative controls were exposed to filtered (0.22 μm) natural sea water (NSW) at 36‰ salinity, pH 8.3 ± 0.1 (N. 1 aquaria). All the aquaria were aerated, water partially renewed (20%) every 24 h and feeding avoided during the experiments. After 4 and 14 day exposure, mussels were collected and tissues sampled for genotoxicity evaluation.

Chemical analysis was performed in duplicate on pooled mussel whole soft tissues (n = 10 specimens *per* pool, weight of each pool = 4.75–5.07 g) following the Method EPA 1613 (US EPA, 1994) and according to Corsolini et al. (2017). Compound determined included 7 PCDD congeners, 10 PCDF congeners, 4 non-*ortho* coplanar PCBs, and 43 PCB congeners, expressed as their sum (Σ43PCBs). The standard solutions used for identification and quantification of single chemicals and for the recovery rate experiments were obtained by Supelco, Inc. (Sigma-Aldrich, U.S.). The limit of detection (LOD) of individual compounds was evaluated as mean blank + 3SD and the values 0.2 (PCBs) ng/g wet weight. Results are given on a dry weight basis (dry wt). The procedure's accuracy (err = 7%) and precision (CV = 5%) were tested through the IAEA-MEL intercomparison exercise for the determination of organohalogen compounds in mussel