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Evaluating the impact of a fluoropolymer plant on a river macrobenthic community by a combined chemical, ecological and genetic approach

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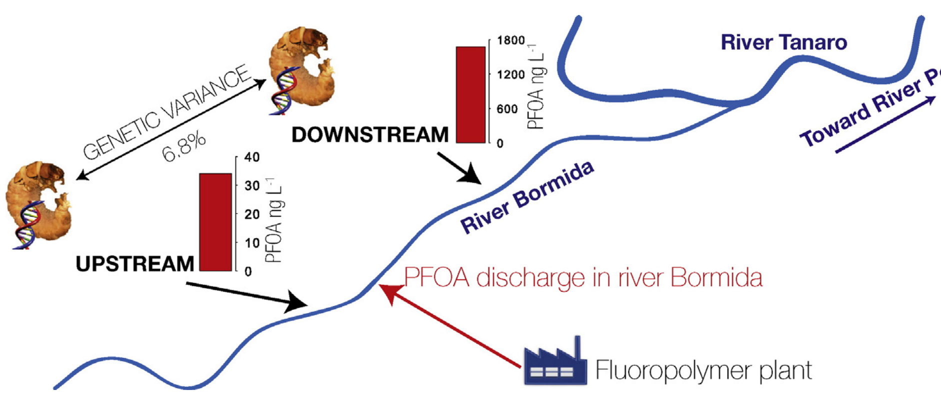
HIGHLIGHTS

- Impact of PFOA from a chemical plant on a river macrobenthic community was assessed.
- Ecological classification did not evidence a clear PFOA impact on benthic community.
- At lower taxonomic level some difference in community composition were evidenced.
- Genetic study identified divergences between PFOA-exposed and unexposed organisms.

GRAPHICAL ABSTRACT



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ABSTRACT

Effect-based monitoring is a recommended approach suggested in European Guidelines to assess the response of ecosystem affected by a pollution source, considering the effects at community, population, individual but also at suborganism level. A combined chemical, ecological and genetic approach was applied in order to assess the impact of a fluoropolymer plant on the macrobenthic community of the Northern Italian river Bormida (Piedmont region). The macrobenthic community living downstream of the industrial discharge was chronically exposed to a mixture of perfluoroalkyl substances (PFAS), with perfluorooctanoic acid as the main compound, at concentrations up to several μg L^{-1}. Ecological assessment proved that the downstream community was not substantially different from that living upstream of the pollution source. The impact on community is not quantifiable with the traditional monitoring methods used for ecological classification under European regulation because macrobenthic communities showed only slight differences in their structure. In order to highlight effects on genetic variability of the native population, a subcellular analysis by using the AFLP (Amplified Fragment Length Polymorphism) genetic technique was applied to genotype of individuals of a selected species (*Hydropsyche modesta*, Trichoptera) collected in the two sampling sites. Percentage of variation between the two populations was 6.8%, a threshold compatible with a genetic drift induced in the downstream population. The genetic study carried out in field identified a significant divergence between exposed and non-exposed populations, but at present it is not possible to associate this divergence to a specific effect induced by PFAS.

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1. Introduction

Since many decades perfluoralkyl substances (PFASs) have been used in a wide range of industrial and consumer products, including surfactants and polymers, mainly to repel dirt, water and oil (Kannan, 2011; Buck et al., 2011; Prevedouros et al., 2006). Scientific studies have been focused mainly on C8-perfluoroalkylacids such as perfluorooctanesulphonic acid (PFOS) and perfluorooctanoic acid (PFOA), which are transported primarily by water but can accumulate in various environmental compartments, because of their peculiar characteristics such as lipophilicity, resistance to hydrolysis, photolysis and microbial degradation (Giesy and Kannan, 2001; Prevedouros et al., 2006).

Fluoropolymer plants are significant sources of poly- and perfluorinated compounds for the aquatic environment (Prevedouros et al., 2006). In particular, plants for the production of polytetrafluoroethylene (PTFE) are the most important source of PFOA, which is used as an emulsifier in aqueous solution during the emulsion polymerization of tetrafluoroethylene. Because PFOA is not consumed during the polymerization process, the remaining compound may be released into air and water (OECD, 2006). In 2006 it was estimated that 60% of the total released PFOA was emitted by fluoropolymer manufacturing and this amount was distributed in air, water, and land for 23%, 65%, and 12% respectively (Prevedouros et al., 2006).

Few studies were carried out to assess the impact of fluoropolymer plants on the surrounding environment, but they generally focused on the distribution of the emitted PFASs into the different environmental compartments, especially air, groundwater and raw water resources (Davis et al., 2007; Dauchy et al., 2012) or neighboring residents blood (Bao et al., 2011). No extensive study was ever designed to unravel the impact of the fluoropolymer plant emission on the aquatic communities. Although perfluoroalkyl acids (PFAA) concentrations in surface waters are generally lower than the predicted no-effect concentrations (PNECs), derived from acute and chronic data for aquatic organisms (Hoke et al., 2012), some PFASs (including the mostly studied PFOS) are suspected to have long-term adverse effects on aquatic biota (Ding and Peijnenburg, 2013; Ahrens and Bundschuh, 2014; Stefani et al., 2014). Moreover, current testing procedures are not generally able to identify sub-lethal effects, such as immunotoxicity and endocrine disruption, which have been linked to PFAS exposure (Scheringer et al., 2014; Grandjean and Budtz-Jørgensen, 2013; White et al., 2011), and little is known about the interactive toxicity of PFASs mixtures at environmentally relevant concentrations or about interactions with other natural and anthropogenic stressors.

In the present paper the impact of the discharge of a fluoropolymer plant on the macrobenthic community of an Italian river was assessed. The plant, located in Spinetta Marengo, next to Alessandria (Piedmont, Northern Italy), discharges into river Bormida, a tributary of river Tanaro, and was recognized as the most significant point source of perfluorooctanoic acid (PFOA) in the river Po basin (Loos et al., 2008). PFOA load from this source was about 2.1 t y^{-1} (Valsecchi et al., 2015) which represented more than 90% of the total load carried out by the river Po to the Adriatic Sea. Riverine benthic community living downstream of the fluoropolymer plant discharge experienced a long term exposition to sub-lethal concentrations of PFAS; this pressure could lead to an impairment in the ecological status which was studied by the combined application of chemical, ecological and genetic analysis in the present work. Potential effects at two different biological levels were considered jointly. First, traditional methods of ecological quality assessment, based on benthic community composition, were applied upstream and downstream of the plant. Second, the toxicological implication at population scale was explored by investigating changes in population genetics of a native benthic organism, with the aim to highlight sub-lethal effects that can accumulate through generations and lead to fitness reduction and, potentially, to population extinction. Genetic erosion, induced by contaminants selection, have been hypothesized to occur even at concentrations of low toxic effect or for low toxic compounds, and this may be of relevance also in the case of PFAS (Bickham, 2011). In fact transgenerational effects of some PFAS at environmental concentrations have been recently demonstrated (Ahrens and Bundschuh, 2014, Stefani et al., 2014). Patterns of altered mutation rates caused by PFOS and PFBS at 10 μg L^{-1} has been evidenced in a 10-generations test with *Chironomus riparius* (Stefani et al., 2014), and it has been hypothesized that long-term effects on population fitness may occur because of mutational load (Bickham et al., 2000; van Straalen and Timmermans, 2002).

Among the possible genetic markers suitable for evolutionary ecotoxicology assessment, we explored the application of Amplified Fragment Length Polymorphism analysis (AFLP) (Vos et al., 1995) in this field case study. One of the main strengths of this technique relies on the possibility to produce a wide genome scan of both selection sensitive and neutral loci in the same time, so to embrace simultaneously all potential issues caused by contaminants on exposed population genetics. The application of AFLP to field case studies, despite challenging, demonstrated that multigenerational exposure to contaminants can induce loss of genetic variability through selection (Bach and Dahllof, 2012; Gardestrom et al., 2008; Paris et al., 2010) and that this may be congruent or precede any toxicological evidence for resistance to novel stressors. Moreover, the presence of potential compensatory effects, such as gene flow from uncontaminated areas, or ecological vicariance with more tolerant species, has been taken into account.

2. Materials and methods

2.1. Study area

The river Bormida is 154 km long with a catchment area of 2609 km^2, which extends between Liguria and Piedmont Regions, and is the main tributary on the right bank of the river Tanaro. Few kilometers after receiving the Bormida waters, the Tanaro flows into the Po, the main river in the Northern Italy.

The studied stretch of the Bormida (Fig. 1) is located at Spinetta Marengo, next to Alessandria (Piedmont Region, Northern Italy) where a fluoropolymer plant is located. The plant, which produces fluoropolymers and fluoroelastomers, is very close to the right bank of the Bormida and discharges the process waters directly into the river after a treatment step (coordinates WGS84: +44° 53′ 33.05″N, +8° 38′ 35.81″E).

The chemical plant was founded in 1905 for the production of superphosphate fertilizers and then for several decades produced also chromates and dichromates. In the eighties the chemical plant was converted to the production of fluorinated polymers. Nowadays the plant produces a wide range of fluorinated specialties including polymers, elastomers, fluids and coatings. Since many years the most important product is polytetrafluoroethylene (PTFE). The Solvay Company signed the 2010/2015 PFOA Stewardship Program to formally stop the environmental release of PFOA and its related compounds within 2015 (US-EPA, 2006) and recently optimized the polymerization processes also testing non-fully fluorinated alternative surfactants, such as the proprietary functionalized polyfluoropolyethers (Wang et al., 2013). Other important products of the plant are perfluoropolyether polymers, sold under the Solvera® and Fluorolink® brand names, whose production capacity has been progressively increased during 2008.

In order to study the impact of the discharge of the fluoropolymer plant, two sampling points were sited along the river up- and downstream of the discharge of the industrial area of Spinetta Marengo (Fig. 1, Table 1). The two sampling sites were 1 km away from each other and were similar in channel width and substrate. Furthermore the same two stations have been used by the Regional Environmental Agency (ARPA Piemonte) for the operational monitoring according to the Water Framework Directive (2000/60/EC, WFD). In the 2009–2011 period the ecological classification was 'moderate' for both

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