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## Influence of global change-related impacts on the mercury toxicity of freshwater algal communities

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### HIGHLIGHTS

- We studied the influence of climate-change drivers on Hg toxicity to periphyton.
- Exposure to Hg under a +5 °C scenario increased its toxicity to algal photosynthesis.
- The algal community and water chemistry modulated temperature effects on Hg toxicity.
- The temperature impacts on river-pollutant toxicity may rely on land-derived materials.

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### ABSTRACT

The climatic-change related increase of temperatures, are expected to alter the distribution and survival of freshwater species, ecosystem functions, and also the effects of toxicants to aquatic biota. This study has thus assessed, as a first time, the modulating effect of climate-change drivers on the mercury (Hg) toxicity of freshwater algal photosynthesis. Natural benthic algal communities (periphyton) have been exposed to Hg under present and future temperature scenarios (rise of 5 °C). The modulating effect of other factors (also altered by global change), as the quality and amount of suspended and dissolved materials in the rivers, has been also assessed, exposing algae to Hg in natural river water or a synthetic medium.

The EC<sub>50</sub> values ranged from the 0.15–0.74 ppm for the most sensitive communities, to the 24–40 ppm for the most tolerant. The higher tolerance shown by communities exposed to higher Hg concentrations, as Jabarrella was in agreement with the *Pollution Induced Community Tolerance* concept. In other cases, the dominance of the invasive diatom *Didymosphenia geminata* explained the tolerance or sensitivity of the community to the Hg toxicity. Results shown that while increases in the suspended solids reduced Hg bioavailability, changes in the dissolved materials – such as organic carbon – may increase it and thus its toxic effects on biota. The impacts of the increase of temperatures on the toxicological behaviour of periphyton (combining both changes at species composition and physiological acclimation) would be certainly modulated by other effects at the land level (i.e., alterations in the amount and quality of dissolved and particulate substances arriving to the rivers).

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### 1. Introduction

The intense use of water resources and the degradation of watersheds have significantly transformed our rivers (Allen and Ingram, 2002; Nilsson and Berggren, 2000; Ward and Stanford, 1995), thus affecting the global water cycle (Beniston et al., 2007; Milly et al., 2005; Rosenzweig et al., 2008). However, these impacts are not the only ones affecting freshwaters; other stressors, such as the climatic-change related increase in temperatures (IPCC, 2014), are expected to

profoundly alter key biogeochemical processes, the distribution and survival of freshwater species or ecosystem functions (Heath et al., 2014; Lorenzo-Lacruz et al., 2012; Winder et al., 2011). In addition to these impacts, pollution as a consequence of industrial, agricultural and urban activities (Kundzewicz et al., 2007) exposes biological communities to a multiple-stressor scenario. Thus, there is an increasing concern for predicting the future impacts of their exposure to the complex exposures scenarios that are created by global change (Petrovic et al., 2011).

Among the most studied and important pollutants in terms of impact on fluvial ecosystems are metals (Admiraal et al., 1999; Hill et al., 2000a; Morin et al., 2008). The toxicity of metals, such as Cu, As, Zn,

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Cd, Pb, Ag, and Hg, has been studied from several approaches, such as lethality, growth rate, bioaccumulation, photosynthetic inhibition and community structure (Corcoll et al., 2012; Guasch et al., 2002; Ivorra et al., 2000; Sabater et al., 2002; Thi Thuy et al., 2010). Particularly, and due to its high toxicity, persistence, and high bioaccumulation potential (Rodrigues et al., 2013), Hg has become one of the most monitored metals in environment, but also in human tissues (Corvelo et al., 2014; Pirrone et al., 2013; Schmid et al., 2013). Coal-fired power plants (emissions), chemical industry (emission and waste disposal) and mining are the main sources of Hg pollution in the environment. The high volatility of Hg facilitates its spread and transport to sites that are remotely located from pollution sources. As example, the Environmental Protection Agency of the U.S. found that more than half of all freshwater fishes sampled from America's lakes contained unsafe levels of Hg for human consumption.

Metal toxicity to aquatic organisms strongly depends on the chemical characteristics of water. For Hg, metal toxicity usually increases as temperature increases; in contrast, high alkalinity tends to decrease the toxicity of Hg. The toxicity of Hg can be reduced by suspended sediments in water (Wang, 1987). The complexity of the modulation of metal toxicity by changes in temperature and water chemistry makes difficult prediction of the effects of metal on aquatic communities. Adaptation of organisms to the presence of pollution, that may change the community response, could be additional factor that makes analyses of relation of biological assemblages and metals more complex and complicate generalisation. Communities growing under stressful conditions (such as those provoked by the presence of Hg in the water) may become more resistant to the effects of Hg. This resistance is based on the rationale of the Pollution-Induced Tolerance Concept – PICT- (Blanck et al., 1988). The PICT indicates that a toxicant has exerted a selective pressure on the community members, provoking a replacement of species or a phenotypic adaptation of individuals (Blanck, 2002) and resulting in a more tolerant community. This phenomenon has been described not only for chemicals but also for physical stressors, such as ultraviolet radiation (Navarro et al., 2008). In this regard, the selected river – Gállego, NE Spain-, presents an industrial hotspot in the middle–upper part that acts as a source of low and chronic levels of Hg (Raldúa, 1995). The presence of Hg in these reaches is expected to be relevant for understanding the periphyton tolerance to Hg. To our knowledge, this study would be the first approach to assess the tolerance of natural freshwater periphyton to Hg.

In this study, the impacts of Hg on three natural freshwater periphyton communities that were exposed to different environmental conditions have been assessed under present temperatures and the predicted temperatures for 2090. The sampling sites are located in the headwaters that are close to the river source, in an upper–middle reach that is exposed to chemical pollution (including Hg) and a lowland region that is exposed to an intense agricultural runoff. Periphyton is an ideal subject for this type of survey because of its physical and physiological characteristics (Dixit et al., 1992; Stevenson, 1999). Future projections indicate that an increase in water temperature and precipitation intensity, combined with longer low flow periods, will boost many sources of materials that will promote the growth of algae, bacteria and fungi (Hall et al., 2002; Kumagai et al., 2003), which are the main components of periphyton. The working hypothesis based on the PICT is that the resistance of periphyton communities to Hg will differ according to the conditions prevailing at each location.

## 2. Material and methods

### 2.1. Study area

The Gállego River is a tributary of the Ebro River, whose course runs through Aragon (NE Spain). This river has a length of 200 km and drains a watershed of 4020 km<sup>2</sup>. The climate in the Gállego River basin presents a gradient from north to south, with 5 different river environments

according to the EU Water Framework Directive (WFD, Directive 2000/60/EC), who proposed two river classification systems (A and B, Annex II) to provide a basis for managing aquatic ecosystems. In Spain, water legislation (ORDER ARM/2656/2008, Ministry of environment) includes an environmental classification (WFD-Ecotypes) based on the system B, which was developed for river segments considered as management units (i.e. those where the definition of environmental flow regimes is mandatory). This hierarchical classification uses seven environmental variables: two hydrologic (annual specific runoff and discharge), three morphological (mean slope and altitude of the watershed, and stream order) and two physicochemical (mean annual temperature and estimated water conductivity).

The annual rainfall is approximately 1330 mm, which is typical of a headwater basin, and 370 mm at its mouth (Fig. 1). Discharges from nearby towns are mostly refined, having a low impact on the overall water quality of the river. However, discharges from past and present industrial activities have a major impact on the middle stretch of the river as it passes through the vicinity of Sabiñánigo. In this area, high levels of pollutants have been detected in the water, sediments and biota from several compounds of Hg, Cr, lindane, HCH, DDT and other substances, with Hg being detected in analyses of biota whose average concentrations over the past 14 years are approximately 1.58 mg/kg, well above the Environmental Quality Standards (EQS) of 20 µg/kg, implying a high rate of bioavailability of Hg compounds (Raldúa, 1995).

To cover the widest diversity of habitats, three sampling sites were selected based on their river position, physicochemical characteristics and anthropogenic pressures (Fig. 1):

#### 2.1.1. Formigal site

This point is located on High Mountain River ecotype (WFD) at an altitude of 1609 m, and its main pressure is the high UV radiation flux. Its physicochemical characteristics are a tranche of oligotrophic nature, with steep gradients, low conductivity (90 µS/cm) and low nutrient levels.

#### 2.1.2. Jabarella site

This point is located on Wet Limestone Mountain ecotype (WFD) at an altitude of 686 m, and its main pressure is the discharge of Hg, organic compounds and other toxic substances from substrate contaminated by historical industrial activity. Its physicochemical characteristics are a stretch of oligo-mesotrophic nature, with a medium–high flow, moderate slopes and medium conductivity (349 µS/cm) and nutrient values.

#### 2.1.3. Violada site

This point is located in a Gallego tributary, very near to the main axis of the river. This site corresponds to a Mediterranean Low Mountain ecotype (WFD) at an altitude of 273 m, and its main pressure is the great burden of runoff from agricultural activities, with occasional high concentrations of terbuthylazine, desethyl-atrazine, metolachlor and chlorpyrifos. This point shows the typical characteristics of eutrophic waters, with a high concentration of nutrients. The presence of saline geological substrates together with the agricultural runoff explains its elevated conductivity (8930 µS/cm).

### 2.2. Colonization

The submerged biofilm was grown on artificial substrates consisting of a flat, heavy rock where 1 or 2 pieces of methacrylate racks with 24 microscope slides were bonded (Fig. 2).

Many studies have demonstrated the significant influence that biomass has on this type of ecotoxicity study (Ivorra et al., 2000; Navarro et al., 2002, 2008). To address the effect of biomass on the toxicity of Hg, biomass accrual was one of the factors that were considered during the toxicity experiments. Periphyton was allowed to develop until presenting enough thickness for measuring at low and high conditions of biomass (see details in Navarro et al., 2008). Due to the differences in

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