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Linking community tolerance and structure with low metallic contamination: A field study on 13 biofilms sampled across the Seine river basin

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ABSTRACT

It is difficult to assess the biological consequences of diffuse water contamination by micropollutants which are present in rivers at low, even sublethal levels. River biofilms, which respond quickly to changes of environmental parameters, are good candidates to acquire knowledge on the response of aquatic organisms to diffuse chemical contamination in the field. The study was designed as an attempt to link biofilm metal tolerance and metallic contamination in a field survey covering 13 different sampling sites in the Seine river basin (north of France) with low contamination levels. Cd and Zn tolerance of heterotrophic communities was assessed using a short-term toxicity test based on β-glucosidase activity. Metal tolerance levels varied between sites but there was no obvious correlation between tolerance and corresponding water contamination levels for Cd and Zn. Indeed, metallic contamination at the sampling sites remained subtle when compared to water quality standards (only two sampling sites had either Zn or both Cu and Zn concentrations exceeding the Environmental Quality Standards set by the EU Water Framework Directive). Yet, multivariate analysis of the data using Partial Least Squares Regression revealed that both metallic and environmental parameters were important variables explaining the variability of metal tolerance levels. Automated Ribosomal Intergenic Spacer Analysis (ARISA) was also performed on both bacterial and eukaryotic biofilm communities from the 13 sampling sites. Multivariate analysis of ARISA fingerprints revealed that biofilms with similar tolerance levels have similar ARISA profiles. Those results confirm that river biofilms are potential indicators of low, diffuse contamination levels of aquatic systems.

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1. Introduction

Over the last decades, several authors have discussed the importance of measuring biological responses at the community level in ecotoxicological studies (Clements and Rohr, 2009; Geiszinger et al., 2009). Indeed, current risk-assessment of toxicants remains largely based on standardized, single-species tests performed in the laboratory, the results of which are then extrapolated at the ecosystem level. Yet, the responses of single-species tests might differ from responses occurring at the community level as important aspects of community ecology are not considered: for instance, interactions between species within the exposed communities are not taken into account (Schmitt-Jansen et al., 2008). Some studies have even suggested that current environmental quality standards might thus not always be sufficient to protect organisms at the community level (McClellan et al., 2008). Moreover, it is generally acknowledged that community endpoints such as species richness and diversity are intimately linked to ecosystem responses to stress and therefore essential to maintain ecosystem services (Clements and Rohr, 2009).

A community-level approach like PICT (Pollution-Induced Community Tolerance) is an interesting ecotoxicological tool to assess the impacts of toxicant exposures (Blanck et al., 2003). The PICT approach proposes to assess shifts in community composition (from a sensitive community to a more tolerant one) due to toxic exposures. It relies on the assumption that sensitive components from the original community (species, genotypes or phenotypes) will be gradually replaced by more tolerant ones during exposure, thus leading to an increase of the global community tolerance. Tolerance development is measured as a shift in the Effect Concentration (usually EC_{50}) or Lethal Concentration (LC_{50}) obtained with a short-term toxicity test based on a physiological endpoint. Tests can be conducted on communities grown in artificial environments (microcosms, mesocosms) or directly on communities collected *in situ*. Interpretation of PICT measurements has proved to be more difficult in field studies either because of co-tolerance, which occurs for chemicals with similar modes of actions or through the development of unspecific defense mechanisms (such as mucilage for algal and bacterial communities) (Schmitt-Jansen et al., 2008; Soldo and Behra, 2000) or because of environmental factors, such as light, nutrients, etc. (Serra et al., 2010; Guasch et al., 2002), which also affect tolerance levels.

However, several PICT studies have investigated river biofilm tolerance to metals or herbicides in the field and succeeded in linking tolerance acquisition to toxic exposure mostly by focusing on one chemical (for instance zinc or atrazine: Blanck et al., 2003; Tlili et al., 2011; Pesce et al., 2010; Admiraal et al., 1999) or more recently on multi-metallic pollution (Fechner et al., 2012a, 2012b), and usually in the same river upstream to downstream from a polluted area. However, field studies attempting to link biological effects and chemical contamination over wide ranges of sampling sites remain scarce. Moreover, current contamination levels are characterized by large numbers of chemicals at low exposure concentrations, which means that for historic contaminants like metals, the issue has shifted from managing acute effects of single toxicants at high exposure levels (which might still occur for instance in areas impacted by mining activities in the case of metals) to managing more subtle, chronic effects of mixtures of chemicals (Schmitt-Jansen et al., 2008). Recent studies, including the PICT studies mentioned above, point out that microbial communities might be good indicators of chemical contamination, even of complex mixtures of chemicals at low exposure levels such as found in the field. Indeed, microbial communities, which undergo fast changes in composition and function in response to changes in environmental parameters, are acknowledged as potential interesting bioindicators of contamination (Sims et al., 2013; Sun et al., 2012; Ricciardi et al., 2009). For instance, Sun et al. (2012) have succeeded in linking variations in microbial community composition in sediment and environmental parameters including sediment metallic contamination using Automated-Ribosomal Intergenic Spacer Analysis (ARISA) data collected over six estuaries sites in Australia. In another recent study, Ancion et al. (2013) also managed to link biofilm-associated metals with variations of bacterial communities using ARISA fingerprints. However, to our knowledge, there are no field studies using tolerance measurements on biofilms from a wide range of sampling sites impacted only by low, diffuse multi-metallic pollution.

The present study was designed as a first attempt at linking metal tolerance and bacterial and eukaryotic community composition of river biofilms to environmental parameters (including physico-chemical parameters and metallic contamination levels) over a wide range of sampling sites in the Seine river basin. Sampling sites were chosen to give a broad representation of contamination levels in a large area impacted by diffuse, low metallic contamination (compared to environmental quality standards). Biofilms were collected at 13 sites in the Seine river basin (North of France) and their Cd and Zn tolerance levels were measured using a short-term toxicity test based on β-glucosidase activity (which measures the tolerance of heterotrophic communities). In parallel, bacterial and eukaryotic community composition was investigated using ARISA. This fingerprinting technique, which exploits the length polymorphism of the 16S–23S intergenic spacer of bacteria and the ITS1-5.8S-ITS2 region of eukaryotes, had already proved useful to assess shifts in community composition upstream to downstream from Paris in the Seine river in previous studies (Fechner et al., 2012a, 2012b). The present study provides a larger survey of river biofilms and their use as possible indicators of urban contamination in a context of diffuse, low and multi-metallic exposure.

2. Materials and methods

2.1. *Collection of river biofilms*

River biofilms were collected at 13 sites located in the Seine river basin (North of France, Fig. 1) after a two-weeks colonization period (see below for details about the collection of biofilm samples).

Sites were sampled over three times from summer (sites 1 to 4 were sampled in early September 2009) to autumn of the