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Review

Combined *in situ* effects of metals and nutrients on marine biofilms: Shifts in the diatom assemblage structure and biological traits

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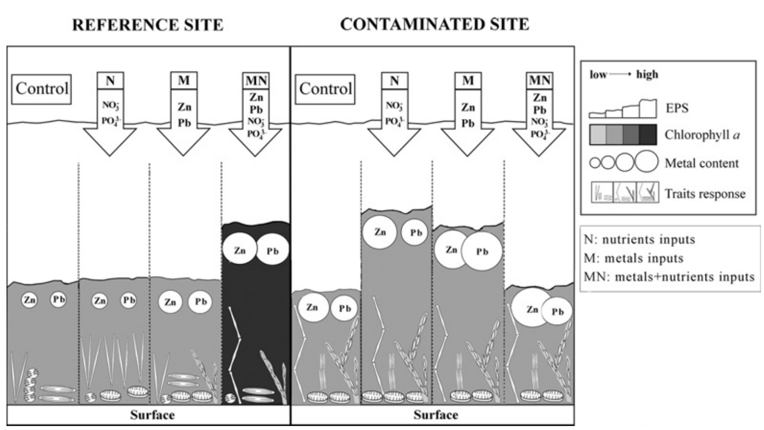
HIGHLIGHTS

- The effects of metal and nutrients depend on the exposure history and diatom composition of biofilms.
- Diatom growth forms and community structures reflect nutrient and metal exposure.
- Nutrients amplify the effects of metal on the community structure and biofilm characteristics in unpolluted sites.
- Metal and nutrient inputs promote a more complex biofilm architecture.
- Biofilms at a chronically contaminated site were more resistant to metal and nutrients inputs.

GRAPHICAL ABSTRACT



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ABSTRACT

The effects of multiple stressors on marine diatom assemblages are still poorly understood. The interactive effects of metals and nutrients were assessed in two coastal biofilms grown at a reference site and a historically contaminated site. The biofilms were exposed in situ to pulse exposures of metals (Zn and Pb) and nutrients (N and P) individually and in combination to mimic patterns of discharge in the study area.

The reference community's structure (composition and abundance of taxa) was modified after metals and/or nutrients exposure, but each stressor acted in different way. Irrespective of the stressors or scenario, the abundance of the dominant species *Opephora krumbeinii* declined, and it is proposed as sensitive species. Nutrient supply favoured the proliferation of certain species with high nutrient tolerances (*Fragilaria famelica*, *Tabularia ktenoeides*), whereas metals promoted the colonisation of metal-tolerant species, e.g., *Berkeleya fennica*, *Opephora marina*. Simultaneous exposure induced an amplification of levels of accumulated metals, chlorophyll *a* and EPS contents and triggered the succession of species towards tolerant species with specific growth. Metals seemed to act as a selective factor of metal-tolerant species, and nutrients favoured the proliferation of those species forming zig-zag colonies (*Neosynedra provincialis*), mucous tubes (*Berkeleya* spp.) and motile diatoms (*Navicula salinicola*, *Nitzschia incognita*), resulting in biofilms with a more complex architecture. The diatom communities from the historically contaminated site were more resistant to pulse exposure, but metals or nutrients loads

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induced overproduction of mucilage. We propose that growth forms may complement taxonomic approaches and provide a quick and easy way to detect community changes related to metal and nutrient pollution.

Contents

1. Introduction

Biofilms are ubiquitous aggregates of organisms composed mainly of bacteria and microalgae embedded in an extracellular polymeric substance (EPS) matrix. In estuarine and shallow systems, diatoms dominate these microalgae benthic communities, which occupy vast extensions of soft-bottom substrates. They play a fundamental role in total primary production (Cahoon, 2006), providing food for other organisms and contributing substantially to geochemical cycles (Blanchard et al., 2000). Biofilms can integrate environmental conditions over long time periods (Dorigo et al., 2010), and the short life cycles of most benthic algal result in a rapid response to environmental pollution, which can be reflected in structural changes to biofilm in a few weeks (Sabater et al., 2007). Of all benthic algae, diatoms are known to be excellent biological indicators and are routinely used to assess pollution impacts (e.g., metals and nutrients) in freshwater systems (i.e., Ivorra et al., 2002; Navarro et al., 2002; Morin et al., 2008b; Tlili et al., 2010, 2011).

Under natural conditions, the responses of the benthic microalgae communities to environmental pollution vary greatly because they depend on the environmental factors and inherent characteristics of biofilms (Navarro et al., 2002). The initial community composition and species interactions can control biofilm responses to individual and mixture of various stressors (Guasch et al., 1998; Breitburg et al., 1999). The wide range of sensitivities among species can generate varied responses to different anthropogenic pressures (Barranguet et al., 2002). Thus, a given toxicant can eliminate or hamper the success of sensitive species and may benefit more tolerant ones through the production of toxicant-induced succession (TIS), which can be observed as changes in the community structure (Blanck, 2002). Nevertheless, natural systems are usually subjected to many human-derived pressures, making it very difficult to predict the impacts of a given toxicant because its effects can be modulated by multiple factors; e.g., the presence of nutrients (e.g., Guasch et al., 2004; Serra et al., 2010). Previous studies have reported different interactive effects of nutrients and toxicants, depending on the type of toxicant. For example, phosphorus (P) can modulate the effects of copper (Cu) on autothrophic communities (Guasch et al., 2004; Serra et al., 2010) but not those of diuron (Tlili et al., 2010). The mixture of substances should also be considered. P compensates for the toxic effects of zinc (Zn) or Cu but not the toxic effects of both metals together (Ivorra et al., 2002). The measured parameters can also provide different interactive results, e.g., functional or structural responses (Tlili et al., 2010; Sundbäck et al., 2007), which can vary among the study system. Most studies on freshwater systems have noted that nutrients can overcompensate metal effects (e.g., Morin et al., 2008a; Guasch et al., 2004; Serra et al., 2010), whereas in marine systems, toxicant impacts seemed to be more evident under nutrient-enriched conditions (Breitburg et al., 1999; Larson et al., 2007). The growth form of species and their microdistribution within biofilms can also determine the responses of benthic communities to metals and nutrients (Ivorra et al., 2002). However, its suitability as an indicator of environmental pollution has been predominantly investigated in freshwater systems for pesticides and other organic pollutants (e.g., Berthon et al., 2011; Rimet and Bouchez, 2011).

Most studies on marine environments have assessed the responses of benthic communities to antifouling biocides (e.g., Blanck et al., 2009; Dahl and Blanck, 1996a), which have shown substance-dependent patterns in community changes (Ohlauson and Blanck, 2014). A few studies have also indicated that metals (Cunningham et al., 2005; Cunningham et al., 2003) or nutrients can modify diatom species composition (e.g., Wachnicka et al., 2011; Armitage et al., 2006; Frankovich et al., 2006). However, very little has been done to study the combined effects of toxicants and nutrients (Larson et al., 2007; Sundbäck et al., 2007) or the ecologic preferences of most species, which precludes the development of biological indicators or tools for biomonitoring in coastal environments (Desrosiers et al., 2013).

The aim of this study was to assess the responses of coastal biofilm communities to pulse exposures of metals and nutrients and their interaction, as well as to investigate the response of biofilms from sites with distinct exposure histories. Biofilms grown in a reference and a historically contaminated site were exposed in situ to metals (100 mg $Zn \cdot L^{-1}$ plus 50 mg $Pb \cdot L^{-1}$), and nutrients (80 μmol $NO^{3-} \cdot L^{-1}$ plus 5 μmol $PO_4^{3-} \cdot L^{-1}$), singly and in combination, simulating the patterns of inputs observed in the study area. The responses of biofilms were investigated on the microalgae community level (diatom community structure and