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Metal concentrations in stream biofilm and sediments and their potential to explain biofilm microbial community structure

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ABSTRACT

Concentrations of metals associated with sediments have traditionally been analysed to assess the extent of heavy metal contamination in freshwater environments. Stream biofilms present an alternative medium for this assessment which may be more relevant to the risk incurred by stream ecosystems as they are intensively grazed by aquatic organisms at a higher trophic level. Therefore, we investigated zinc, copper and lead concentrations in biofilms and sediments of 23 stream sites variously impacted by urbanisation. Simultaneously, biofilm bacterial and ciliate protozoan community structure was analysed by Automated Ribosomal Intergenic Spacer Analysis and Terminal Restriction Fragment Length Polymorphism, respectively. Statistical analysis revealed that biofilm associated metals explained a greater proportion of the variations observed in bacterial and ciliate communities than did sediment associated-metals. This study suggests that the analysis of metal concentrations in biofilms provide a good assessment of detrimental effects of metal contaminants on aquatic biota.

1. Introduction

Metals are naturally present in aquatic ecosystems as a result of weathering of soil and rocks (Bradl, 2005). However, whereas low concentrations of some trace metals can occur naturally in freshwater, anthropogenic activities like industry and mining (Bradl, 2005; Douglas and Beveridge, 1998) as well as urbanisation (Chalmers et al., 2007; Ward and Trimble, 2003) have considerably increased metal concentrations in surface waters. In urban dominated catchments, trace metal concentrations are generally several times higher than background levels (Rice, 1999; Rose and Shea, 2007) and may result in significant damage to ecosystems (Brix et al., 2011; Kiffney and Clements, 2003).

Metal ions bind easily with suspended particles such as silt or organic matter. Depending on the characteristics of each element and on the amount and the nature of particles transported by the water, a significant proportion of the contaminants can be transported in association with particles (Bibby and Webster-Brown, 2006). As sediments are formed by the particles settling out of the water column, they accumulate contaminants associated with the particles and constitute an integrative measurement of the contaminants present in the water. Therefore, to assess whether heavy metals are present in the ecosystem at concentrations that are detrimental to aquatic biota, sediments are commonly analysed for metal concentrations (ANZECC and ARMCANZ, 2000; USEPA, 2005). Although less frequently used, stream biofilms present an alternative medium for the assessment of metal contamination (Mages et al., 2004; Ramelow et al., 1987).

Stream biofilms are a complex aggregation of microorganisms embedded in a polymer matrix and cover almost every surface in freshwater environments. Although biofilms are constantly growing to regenerate biomass that has been grazed or stripped off by the water flow, they are relatively sessile structures and constitute a potential integrative indicator of water contamination. Biofilms retain suspended particles and may therefore accumulate particulate contaminants, as do sediments. However, concentrations of trace metals associated with freshwater biofilms and stream bed sediments can differ significantly (Farag et al., 2007, 1998; Holding et al., 2003), suggesting differences in the affinity of biofilms and sediments for metals. Indeed, biofilms have various properties that may promote the binding of metal ions. The extracellular polymeric substances (EPS) exuded by biofilm microbial communities include ionisable functional groups that enable the fixation of metals (Jang et al., 2001; van Hullebusch et al., 2003), whilst microbial cells provide a vast surface area with an overall anionic charge ideal for binding metals (Douglas and

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Beveridge, 1998). Biofilm can also immobilize metals by precipitation of metal complexes (Douglas and Beveridge, 1998; Wuertz et al., 2000).

Within stream food webs, biofilms are intensively grazed by protozoa, benthic invertebrates and fish. Therefore, the binding and the accumulation of metals in biofilms could play an important role in their transfer to higher trophic levels, as suggested by the strong correlation between metal concentrations in biofilms and in aquatic invertebrates (Farag et al., 2007; Rhea et al., 2006). As aquatic organisms are affected by metal concentrations not only in their surrounding water but also in their food (Clearwater et al., 2002; Courtney and Clements, 2002), analyses of metal associated with biofilms may constitute a more relevant indicator of the potential threat to aquatic organisms than analyses of metal associated with sediments.

The objectives of the present study were therefore (i) to compare concentrations of metal associated with biofilms and with sediments, and (ii) to evaluate whether they provide a good assessment of the potential detrimental effects of metal on aquatic organisms. To do so, we measured concentrations of metals associated with epilithic biofilms and sediments of 23 highly characterised New Zealand stream sites. We focused specifically on zinc, copper and lead because of their common abundance in urban runoff (Chalmers et al., 2007; Kayhanian et al., 2007) and their environmental significance in aquatic environments (Ancion et al., 2010; Mance, 1987).

To evaluate the effectiveness of metal concentrations measured in biofilm or in sediments in providing an assessment of potential impairments to freshwater ecosystems, the relationship between metal concentrations and the responses from the ecosystem was investigated. Communities of macro-invertebrates (Boothroyd and Stark, 2000), fish (Walters et al., 2009), macrophytes (Thiebaut et al., 2002) algae (Dokulil, 2003) or diatoms (Wu, 1999) are commonly used as biological indicators of stream environmental conditions. Alternatively, biofilms are complex assemblages of microorganisms that can also provide a community level perspective on biological responses to environmental disturbances. The use of microbial communities as biological indicators was limited in the past by the lack of methodological tools but molecular fingerprinting techniques now provide a rapid and cost effective method to screen a large number of samples (Theron and Cloete, 2008). Microorganisms associated with biofilms can thus be used to monitor the impact of catchment land use activities on streams (Dopheide et al., 2008; Lear et al., 2009) with the advantage of being widely applicable because of the ubiquity of biofilms in freshwater environments. Microorganisms also respond rapidly to changes in their environment due to their short generation time (Paerl and Pinckney, 1996).

In this study, we used two DNA fingerprinting techniques, Automated Ribosomal Intergenic Spacer Analysis (ARISA) and terminal Restriction Fragment Length Polymorphism (T-RFLP) to respectively characterise bacterial and ciliate protozoan communities associated with biofilms. The relationship between differences in microbial community structure and concentrations of zinc, copper and lead in both stream biofilms and sediments was then investigated to determine if both media provide a useful assessment of the likely impacts of metals on stream ecological health.

2. Material and methods

2.1. Study sites

A total of twenty-three stream sites were identified across the Auckland Region (Fig. 1) to cover all the major catchment types, from native forest to exclusively urban catchments. Consequently, these sites were exposed to a wide range of physical and chemical parameters and to various potential sources of trace metal contamination, including farming, residential areas and industry. The sites examined in this study are part of a long-term water quality monitoring program led by the National Institute of Water and Atmospheric Research (NIWA, New Zealand) for the Auckland Regional Council (ARC, New Zealand). Trends in water quality for these sites are available and provide information about catchment land use, water temperature, conductivity, pH, turbidity, ammoniacal nitrogen, nitrite and nitrate, total phosphorus, dissolved reactive phosphorus and dissolved oxygen (ARC, 2007). The main characteristics of the stream sites are summarized in Table 1.

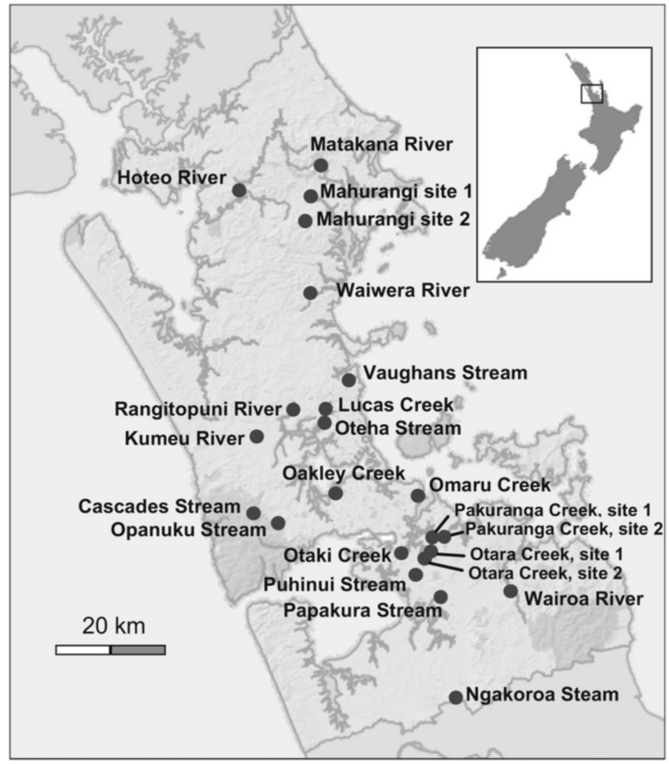

Fig. 1. Location of sampling sites around the Auckland Region, New Zealand. More details about site locations can be found in ARC (2007). Mahurangi: site 1 = at Warkworth water treatment plant, site 2 = at Forestry HQ, Pakuranga: site 1 = at Greenmount Drive, site 2 = at Guy's Rd, Otara: site 1 = at Kennel Hill, site 2 = at East Tamaki Rd.

2.2. Sampling procedure

All sites were sampled over one week (08.01.2008–11.01.2008). At each site, samples of both biofilm and sediments were obtained within a 30 m section of the stream. Biofilm was sampled from three rocks for metal analysis and from five rocks for molecular analysis. Alternatively, when too few rocks were available, a minimum of three rocks were sampled for molecular analysis. For both molecular and metal analysis, biofilm sample was scraped off an individual rock from an area of approximately 100 cm^2 using a new Speci-Sponge™ (Nasco, U.S.A.). The Speci-sponge™ was then placed into an individual Whirl-Pak™ bag (Nasco, U.S.A.) and transported to the laboratory in darkness on ice. Sediments were obtained from each site by collecting three samples of 30 cm^3 from the top two centimetres of the stream bed using a single use plastic scoop and stored in 50 ml Falcon™ tubes (BD Biosciences, U.S.A.). At each site, rock and sediment samples were not collected in a uniform manner, but based on the availability of the substrates at each location.

2.3. Analysis of metal concentrations and composition of biofilm and sediment samples

In the laboratory, each sponge sampled for metal analysis was transferred to an individual stomacher bag (Seward, UK) containing 20 ml of sterile Milli-Q™ water and macerated for 120 s at maximum speed with a stomacher (Stomacher 400, Seward, UK). Sponges were then squeezed to expel the biofilm and the resulting biofilm suspension was filtered through a 250 μm mesh nylon filter to remove coarse fractions (e.g. leaf detritus, moss or little rocks). This suspension was transferred to an individual 250 ml Teflon® beaker, previously acid-washed and weighed ($\pm$0.05 mg) and dried at 55 °C until no change in weight could be detected within 24 h. Beakers were then re-weighed ($\pm$0.05 mg).