Effects of stormwater metal contaminants on microbial communities in stream biofilms revealed by Automated Ribosomal Intergenic Spacer Analysis (ARISA).

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Stream Biofilm
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Abstract:

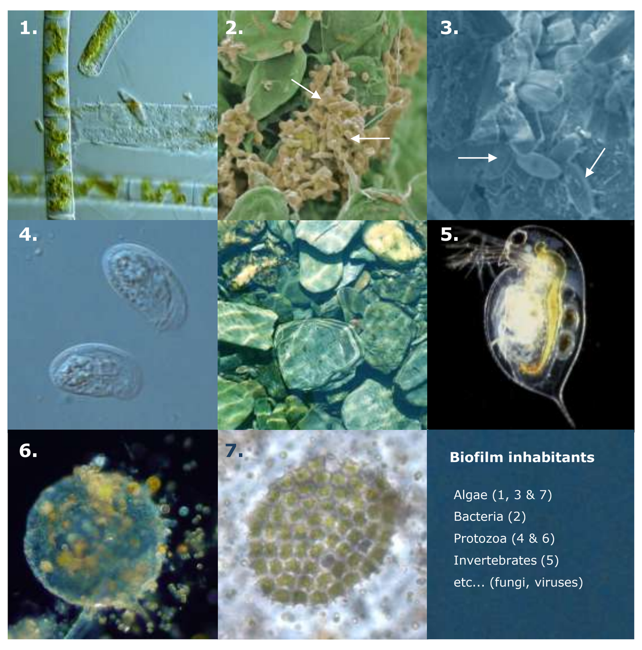
Stormwater metal contaminants are known to be a threat to our freshwater environments but little is known about their effects on stream micro-organisms. This project investigates accumulation and release of the most common stormwater metal contaminants (zinc, copper and lead) in stream biofilms and their effects on bacterial populations.

Stormwater and its effects on stream ecosystems:

Urbanisation is known to have considerably increased pollutant concentrations in surface waters. Metals are among the most common contaminants in stormwater and they frequently constitute a threat to our aquatic ecosystems. So far, much research has focused on the effects of metals on aquatic macro-organisms, especially fish and benthic invertebrates, but little is known about their effects on stream micro-organisms.

What are stream biofilms ?

A stream biofilm is the layer of greenish-brown slime that is found on rocks, plants and other surfaces in a stream. This layer is composed of a wide range of microscopic organisms such as bacteria, algae and protozoa embedded in a complex polymer matrix. Biofilms are intensively grazed by benthic invertebrates and fish and therefore play an important role as a source of nutrients for other organisms. Biofilms could also play an important role in metal immobilization and in the transfer of metals to organisms at higher trophic levels.



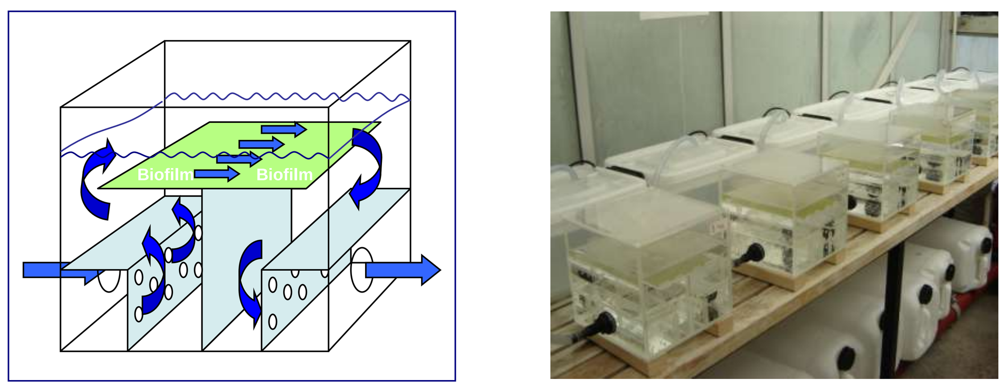
The complex assemblage of a biofilm.

Research aims:

1. To determine the accumulation rate of stormwater metals in biofilms
2. To reveal the effects of this accumulation on bacterial populations.

Experimental design:

Experiments were undertaken in flow chamber microcosms, designed to reproduce natural stream conditions.



Flow chamber microcosms: a water pump makes the water flow above the biofilm to simulate a natural stream flow.

Mature biofilms were exposed to environmentally relevant concentrations of zinc, copper and lead for different periods of time and eventually brought back to clean water to monitor the recovery of populations (see table 1).

Table 1: Metal concentrations in the different treatments and sampling times

Experiment	Treatments	Concentrations (µg/l)	Sampling times
short term	Low	Zn 500 + Cu 50 + Pb 50	1 day, 3 days, 5 days
	High	Zn 1000 + Cu 100 + Pb 100	
long term	Low	Zn 500 + Cu 50 + Pb 50	7 days, 14 days, 21 days
	High	Zn 1000 + Cu 100 + Pb 100	
recovery	Low	Zn 500 + Cu 50 + Pb 50	7 days, 14 days, 21 days
	High	Zn 1000 + Cu 100 + Pb 100	

Treatment
Recovery

Three experiments were conducted successively: a short term experiment (biofilm sampled after 1, 3 and 5 days of exposure), a long term experiment (biofilm sampled after 1, 2 and 3 weeks of exposure) and a recovery experiment (biofilm sampled after 1 week of treatment and then after 1 and 2 weeks of recovery in clean water)

Following exposure, biofilm was carefully removed from the flow chambers, observed under a microscope and sampled for metal and bacterial community analysis. Uptake of metals from water to biofilms was revealed by atomic adsorption spectrometry (FAAS & GFAAS). Bacterial communities were assessed using a molecular community analysis technique (ARISA).

Acknowledgments:

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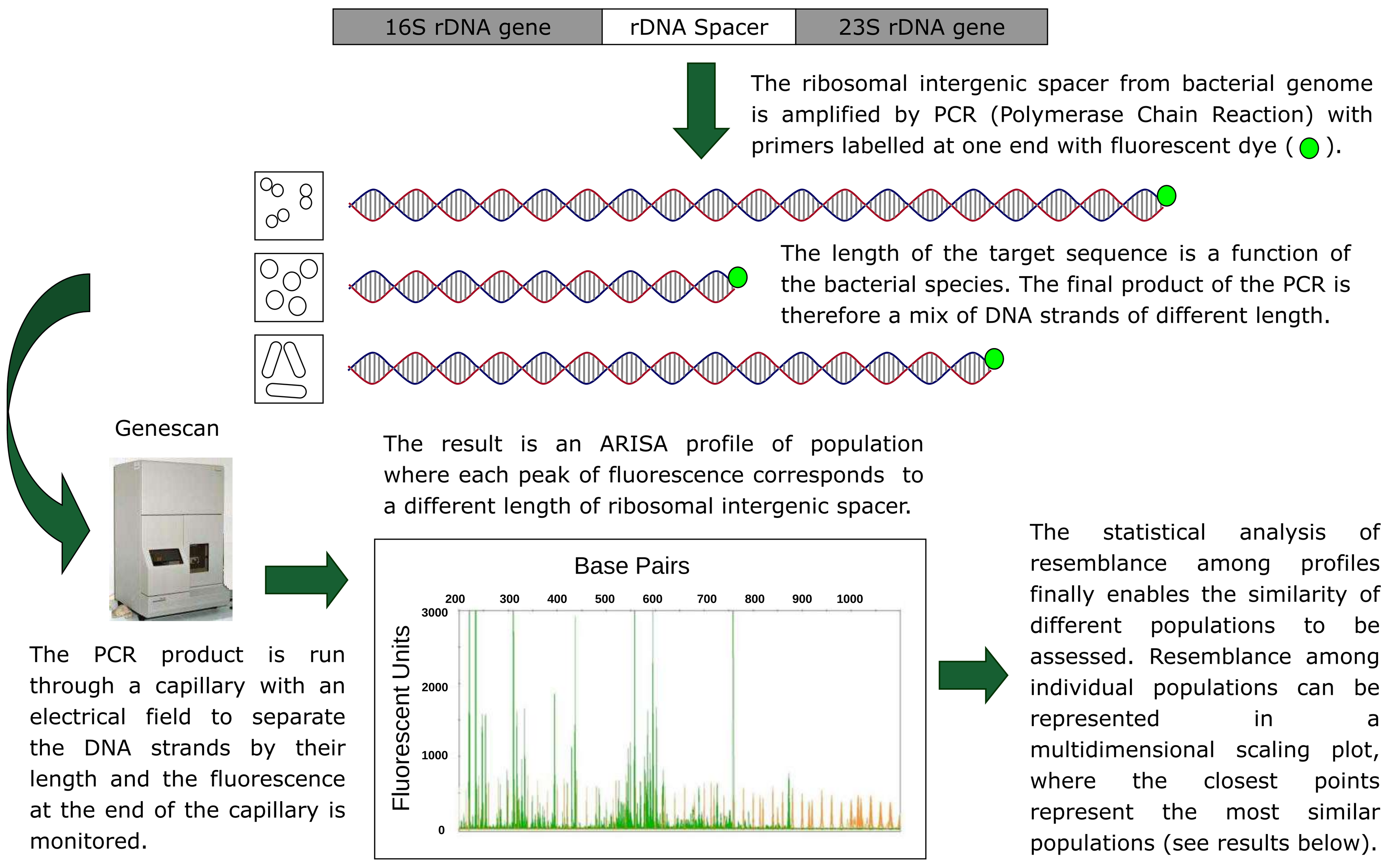
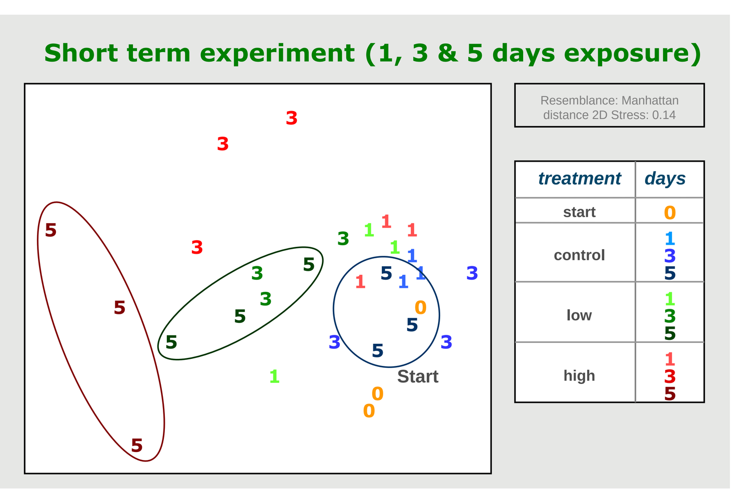
ARISA, a tool to observe bacterial populations:

What happens in complex assemblages such as freshwater biofilms following environmental changes remains poorly understood mainly because of the difficulty of studying microbial populations. Automated Ribosomal Intergenic Spacer Analysis (ARISA) is particularly suited to the statistical analysis of similarity and structure of bacterial communities. It is based on the fact that in the bacterial genome, the DNA sequence between the genes coding for the two ribosomal subunits (16s & 23s) varies in length between different bacterial species (for method, see figure below).

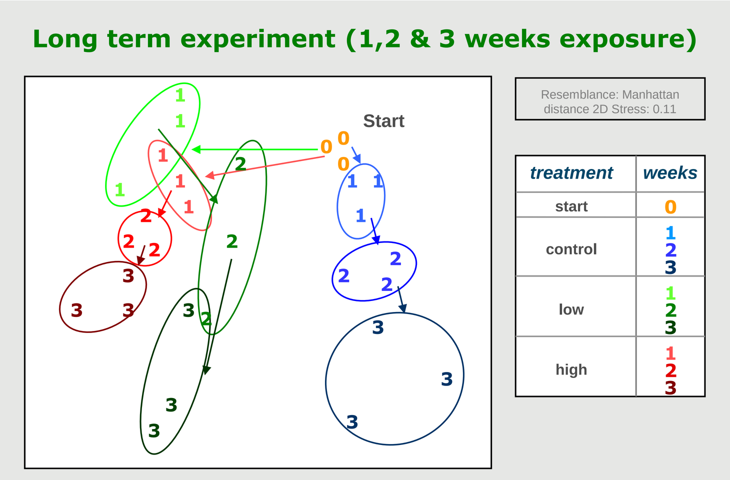


Results: A. Changes in bacterial populations

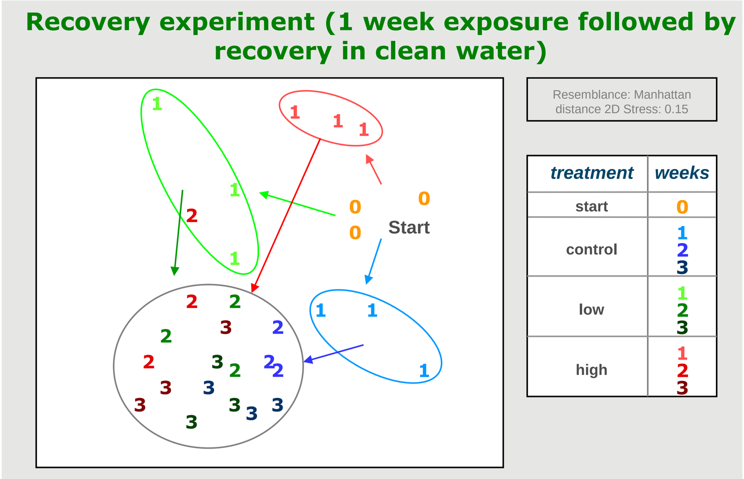
Multidimensional scaling (MDS) shows large shifts in bacterial populations between both treatments and the control. The shift became apparent after 3 to 5 days (short term experiment) and increased until the longest exposure period of 3 weeks (long term experiment). After the biofilm was returned to clean water, the populations impacted by metal exposure changed quickly to become more similar to the control one (recovery experiment). These results reveal that bacterial populations are highly sensitive to metal concentrations and suggest an adaptation towards metal tolerant communities.



MDS plot of similarity, short term experiment



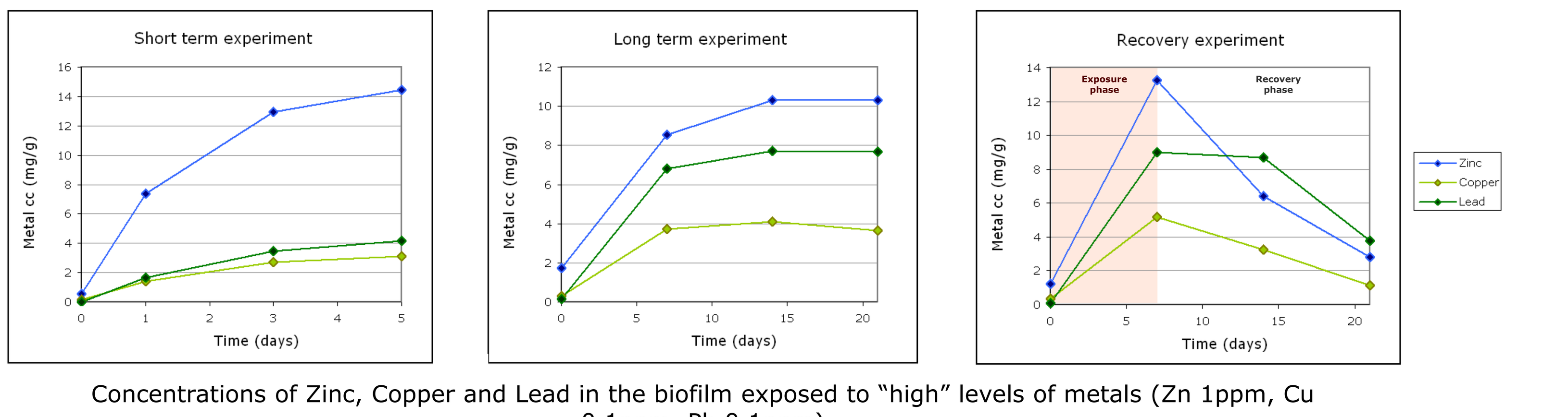
MDS plot of similarity, long term experiment



MDS plot of similarity, recovery experiment

B. Accumulation / release of metals

Metal analysis by Atomic Absorption Spectrometry (AAS) show a quick accumulation of all three metals in the first 5 days followed by a stabilization at around 10 to 15 days. Metal release after replacement in clean water is slower, taking more than two weeks to reach the initial concentrations.



Concentrations of Zinc, Copper and Lead in the biofilm exposed to "high" levels of metals (Zn 1ppm, Cu 0.1ppm, Pb 0.1ppm)

Conclusions:

1. This project demonstrates the successful and cost efficient use of a molecular biology technique (ARISA) to assess the changes occurring in biofilm bacterial populations.
2. ARISA results showed significant shifts in bacterial community profile in response to metal treatment and the development of metal tolerant communities.
3. The rapid accumulation of metals in biofilms during treatment underlines the potential role biofilms could play in the transfer of metals to higher trophic levels such as protozoa, benthic invertebrates and fish which graze microbial slime.