

Biofilms In Ornamental Waters: A Risk Assessment Study

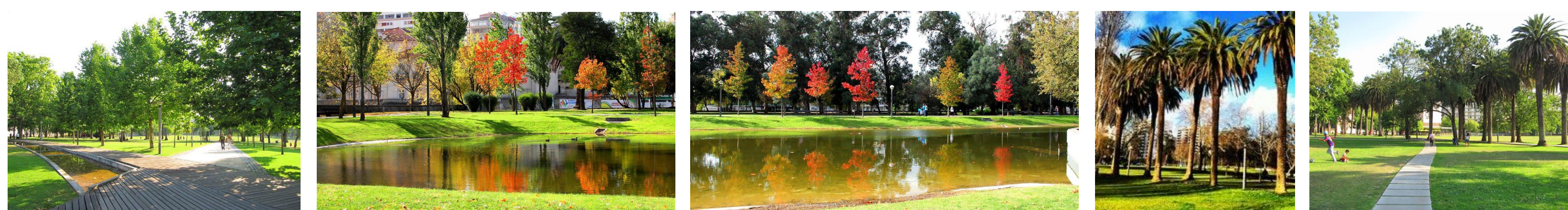
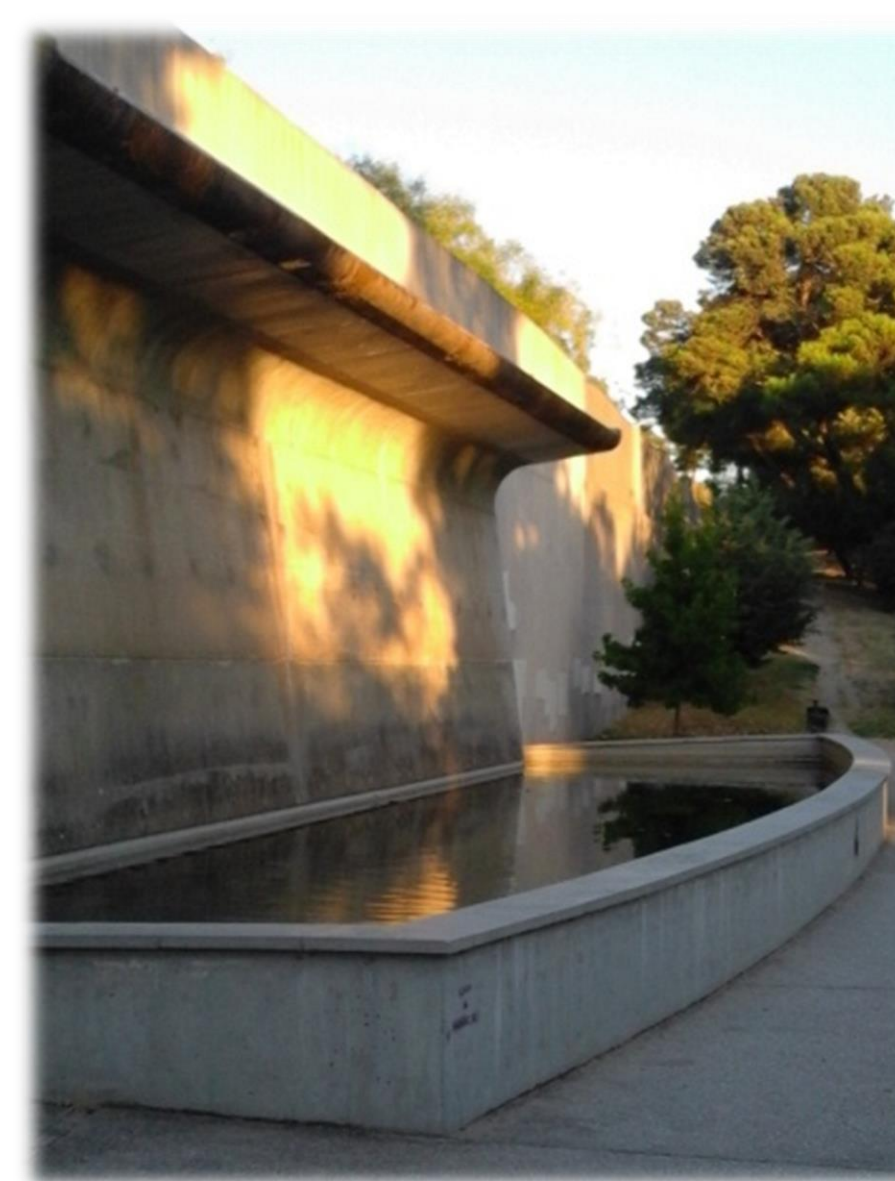
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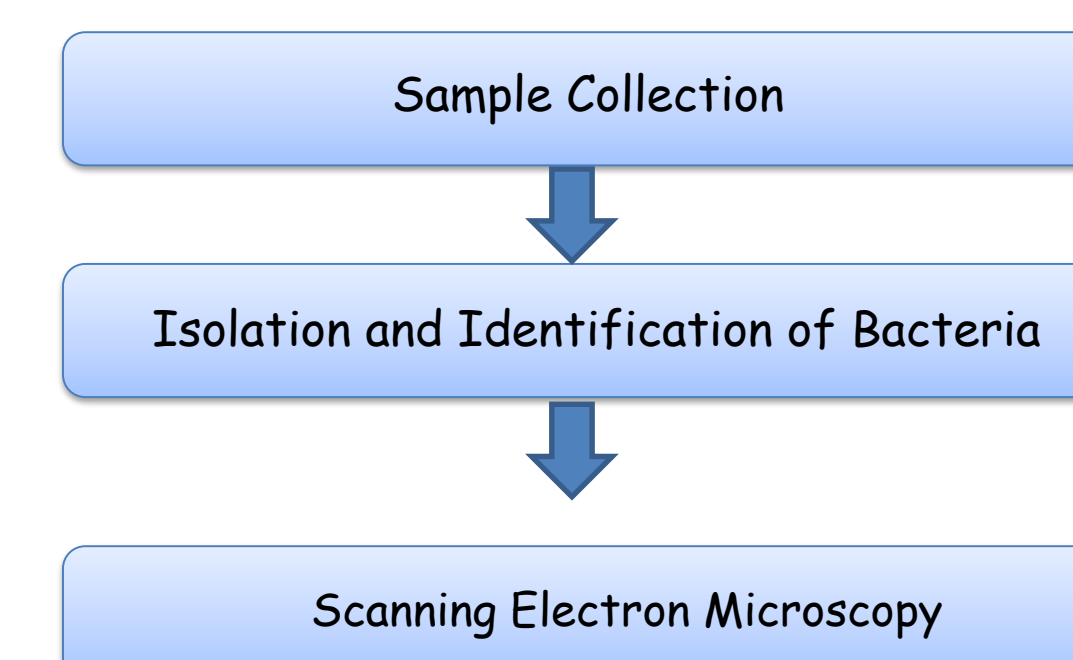
Introduction

Ornamental waters of easy access and populated with animals are quite attractive but can hide threats to human health. Several studies have provided evidence for the presence of potential human pathogens in waters containing ornamental fish. Here we evaluated, during one year, the water chemical composition and the microbiota of an ornamental fountain in a Lisbon park in order to assess the risk for human health.



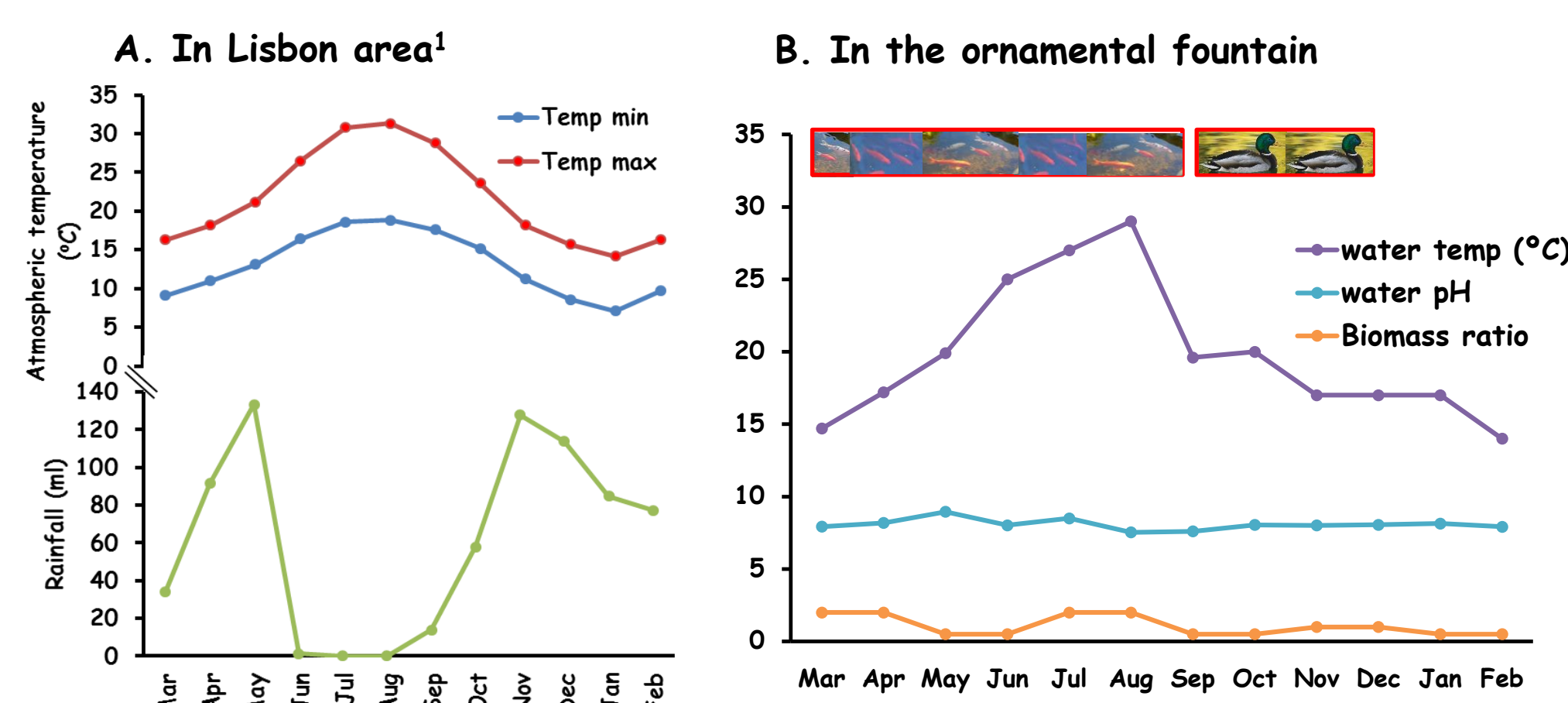
Materials and Methods

Water and biofilm samples were collected once a month from March 2016 to February 2017. Bacterial identification and biofilm analysis were performed as described before² following the workflow below.



Results:

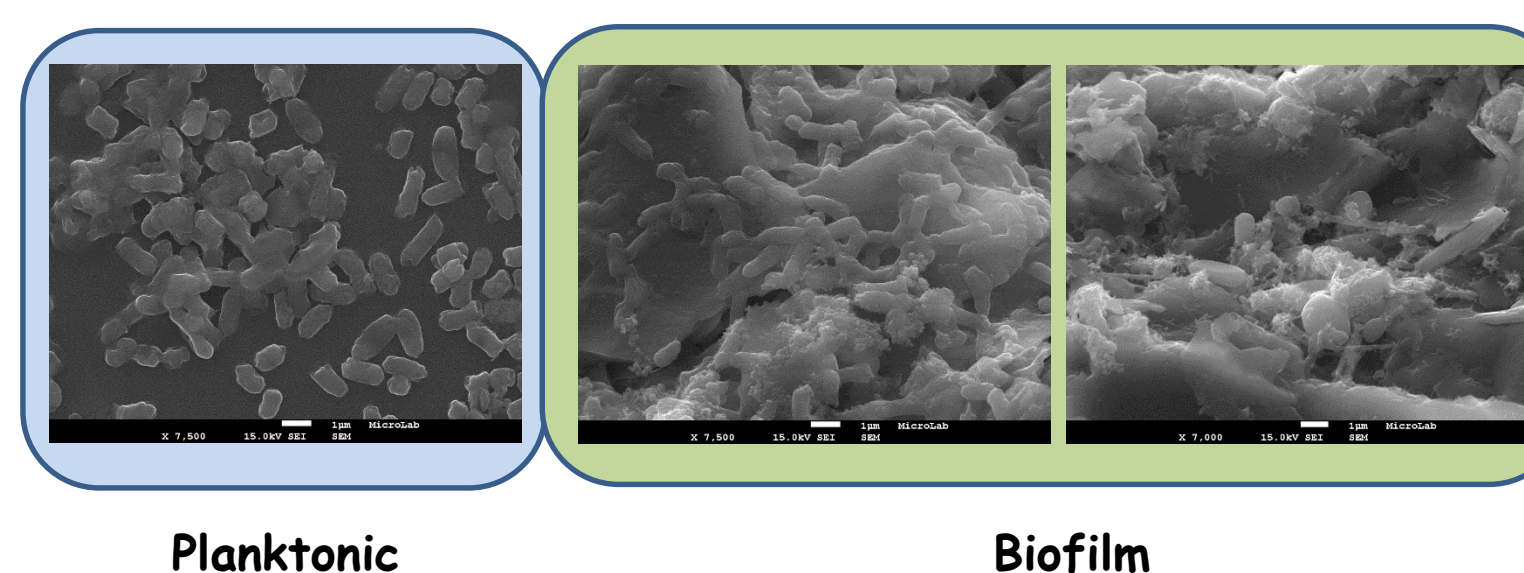
1. General features.



26 different bacterial species were identified including potential human pathogens such as *Aeromonas*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas* and *Raoutella*.

Aeromonas sobria is a resident bacterium whereas other species such as *A. hydrophila* and *A. veronii* were sporadically identified. *Pseudomonas fluorescens*, *P. putida* and *P. oryzihabitans* have been considered low-virulence bacteria but recently have been identified as ethiological agents of healthcare associated infections (HAI) mostly in immunosuppressed individuals. Another recognized HAI agent present is *K. pneumoniae*.

3. Biofilms <-> Planktonic bacteria.



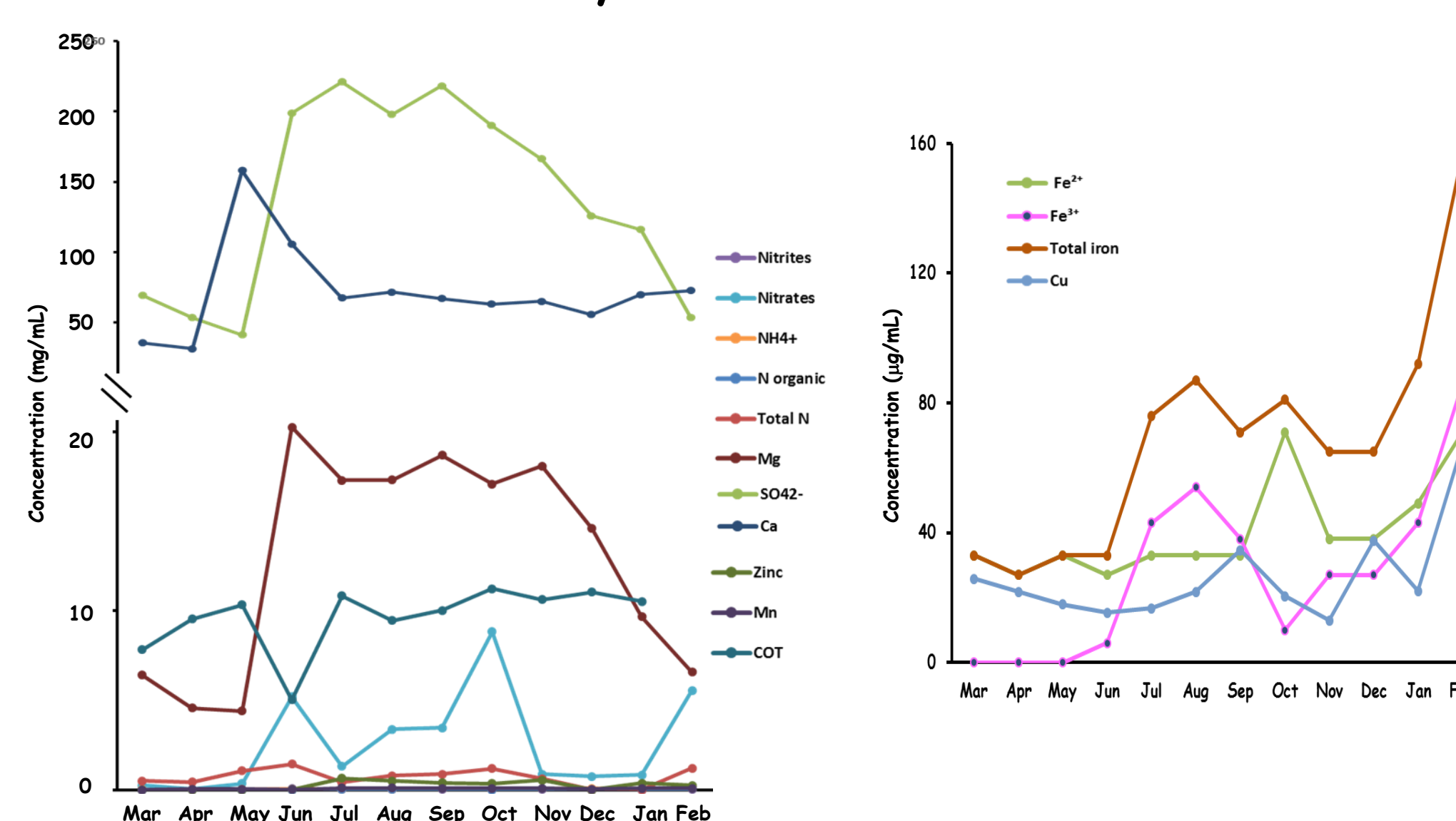
The same bacterial species were isolated from biofilm and water suggesting the existence of an equilibrium between planktonic and biofilm organized bacteria.

2. Microbiota.

Bacteria	2016												2017		
	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	
<i>Actinobacter lwoffii</i>															
<i>Aeromonas hydrophila/caviae</i>															
<i>Aeromonas sobria</i>															
<i>Aeromonas veronii</i>															
<i>Alcaligenes faecalis spp faecalis</i>															
<i>Bacilo Gneg</i>															
<i>Bacillus subtilis</i>															
<i>Comamonas testosteni/ P oleovorans</i>															
<i>Enterobacter cloacae complex</i>															
<i>E coli</i>															
<i>Escherichia fergusonii</i>															
<i>Klebsiella oxytoca</i>															
<i>K pneumoniae spp pneumoniae</i>															
<i>Lectercia adecarboxylata</i>															
<i>Pantoea spp</i>															
<i>Pseudomonas fluorescens</i>															
<i>Pseudomonas oryzihabitans</i>															
<i>Pseudomonas putida</i>															
<i>Pseudomonas spp</i>															
<i>Raoutella ornithinolytica</i>															
<i>Raoutella planticola</i>															
<i>Serratia fonticola</i>															
<i>Serratia marcescens</i>															
<i>Serratia plymuthica</i>															
<i>Shewanella putrefaciens</i>															
<i>Shingomonas paucimobilis</i>															

Microorganism isolated from the water (□) or biofilm (■)

4. Water: Chemical analysis.



The chemical composition of superficial water samples was also determined. Spectrophotometric methods were used to determine nitrites (ISO 6777:1984), nitrates, NH₄⁺ (ISO 7150/1), total N, SO₄²⁻, Zn, Mn (ISO 6333:1986), total organic carbon (COT), Cu, Fe²⁺ and total iron (ISO 6332:1988). Calcium was determined using a volumetric method according to the NP506. Fe³⁺ and organic N were calculated subtracting Fe²⁺ from total Fe and subtracting the NH₄⁺ from total N, respectively. pH was assessed by potentiometry.

References

- 1, IPMA, Boletins climatologicos mensais (Mar2016- Feb 2017)
- 2, Nascimento M et al., Pathogens in Ornamental Waters: A Pilot Study. *Int J Environ Res Public Health*. 2016 Feb 15;13(2):216. doi: 10.3390/ijerph13020216.

Conclusions

- In this study potential human pathogens were identified in samples from a typical urban park.
- These pathogens were found in both planktonic and biofilm forms.
- Waterborne pathogens such as *Aeromonas* species are a significant cause of acute bacterial gastroenteritis in young children.
- The obtained results support a periodic control of ornamental water microbiota as simple preventive measure to avoid potential health issues.