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Enrichment of free-living amoebae in biofilms developed at upper water levels in drinking water storage towers: An inter- and intra-seasonal study

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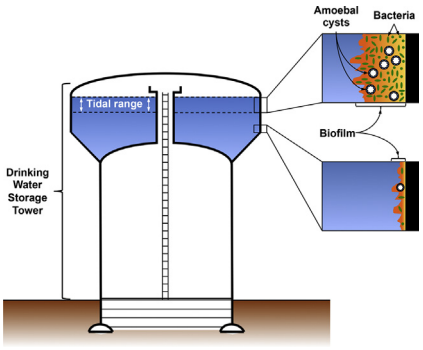
HIGHLIGHTS

- High amoebae density at the surface of drinking water storage towers
- Seasonal evolution of amoebae in drinking water storage towers
- Presence of *Acanthamoeba* in two spring sampling campaigns

GRAPHICAL ABSTRACT



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ABSTRACT

Free-living amoebae (FLA) are ubiquitous organisms present in various natural and artificial environments, such as drinking water storage towers (DWST). Some FLA, such as *Acanthamoeba* sp., *Naegleria fowleri*, and *Balamuthia mandrillaris*, can cause severe infections at ocular or cerebral level in addition to being potential reservoirs of other pathogens. In this work, the abundance and diversity of FLA was evaluated in two sampling campaigns: one performed over five seasons in three DWST at three different levels (surface, middle and bottom) in water and biofilm using microscopy and PCR, and one based on the kinetics analysis in phase contrast and confocal microscopy of biofilm samples collected every two weeks during a 3-month period at the surface and at the bottom of a DWST. In the seasonal study, the FLA were detected in each DWST water in densities of ~20 to 25 amoebae L^{-1}. A seasonal variation of amoeba distribution was observed in water samples, with maximal densities in summer at ~30 amoebae L^{-1} and minimal densities in winter at ~16 amoebae L^{-1}. The FLA belonging to the genus *Acanthamoeba* were detected in two spring sampling campaigns, suggesting a possible seasonal appearance of this potentially pathogenic amoeba. Interestingly, a 1 log increase of amoebae density was observed in biofilm

Abbreviations: DWDS, Drinking water distribution system; DWST, Drinking water storage tower; FLA, Free-living amoebae; AK, *Acanthamoeba* keratitis; GAE, Granulomatous Amoebic Encephalitis; BDOC, Biodegradable dissolved organic carbon; TOC, Total organic carbon.

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samples collected at the surface of all DWST compared to the middle and the bottom where FLA were at 0.1–0.2 amoebae/cm^2. In the kinetics study, an increase of amoebae density, total cell density, and biofilm thickness was observed as a function of time at the surface of the DWST, but not at the bottom. To our knowledge, this study describes for the first time a marked higher FLA density in biofilms collected at upper water levels in DWST, constituting a potential source of pathogenic micro-organisms.

1. Introduction

Free-living amoebae (FLA) are widely distributed protozoa in nature that could be found in many natural and artificial environments including rivers, soil, swimming pools and drinking water distribution systems (DWDS; Rodriguez-Zaragoza, 1994; Visvesvara et al., 2007; Thomas and Ashbolt, 2011). Most FLA develop autonomously without the need of a host (Rodriguez-Zaragoza, 1994). However, some of them, especially *Naegleria fowleri*, *Balamuthia mandrillaris* and those belonging to the *Acanthamoeba* genus, are amphizoic which develop as free-living organisms in the environment or as opportunistic pathogens within a host (Visvesvara et al., 2007). These FLA can cause diverse pathologies with main tropisms in the eye or the brain. At the ocular level, *Acanthamoeba* sp. can cause the *Acanthamoeba* keratitis (AK), which is a sight-threatening infection affecting mainly contact lens wearers. At the cerebral level, *Acanthamoeba* sp. and *Balamuthia mandrillaris* are responsible of Granulomatous Amoebic Encephalitis (GAE), and *Naegleria fowleri* can provoke Primary Amoebic Meningoencephalitis (PAM), displaying a mortality rate superior to 90% (Schuster and Visvesvara, 2004; Visvesvara et al., 2007). In addition, FLA can host diverse microorganisms, such as bacteria, viruses, fungi or protozoa, and constitute a potential reservoir of human pathogens (Guimaraes et al., 2016; Thomas et al., 2010; Winiecka-Krusnell et al., 2009). Therefore, due to their own pathogenic properties and to their ability to disseminate potential infectious microorganisms, FLA constitute a potential risk for human health.

The FLA display mainly two stages in their life cycle: the trophozoite, a vegetative form which can invade and damage host tissues, and the cyst, a dormant resistant form which allows the FLA to persist in hostile conditions and to propagate in various environmental niches. Some FLA cysts have been described to resist to different disinfection methods used to treat drinking water, such as chlorine, ultraviolet radiations, ozonation or filtration (Cervero-Arago et al., 2014; Siddiqui et al., 2008; Storey et al., 2004; Thomas et al., 2004). In particular, FLA can develop in biofilms generated in DWDS, allowing a rapid colonization of the network in case of treatment interruption (Thomas et al., 2004). These biofilms would favour the contact between FLA and bacteria, and would thus constitute a major source of amoeba-resisting microorganisms in drinking water (Thomas et al., 2008).

Although ecology of FLA has been studied in DWDS (Thomas and Ashbolt, 2011), few studies were performed in drinking water storage towers (DWST; Amblard et al., 1996; Lu et al., 2015). In particular, an increase of amoebae number was observed in DWST compared to the inflowing water, suggesting that DWST would constitute a favourable environment for the development of these microorganisms (Amblard et al., 1996).

In this work, the presence of FLA was evaluated by analyzing their abundance and diversity over 5 seasons in water as well as in biofilm samples of three different DWST. The kinetics of FLA development was also investigated during biofilm formation. These seasonal and kinetics studies allowed characterizing temporal and spatial FLA distributions in DWST, in comparison with analyses performed 20 years ago in the same drinking water network (Amblard et al., 1996). In particular, the presence of potential pathogenic amoebae was investigated to evaluate the health risk associated with FLA in DWST.

2. Materials and methods

2.1. Physico-chemical analyses

Temperature, turbidity and dissolved oxygen concentration were measured *in situ* for each sample using Temp5 thermometer (Ecoscan, Fisher, Courtaboeuf, France), 2100P turbidimeter (Hach, Düsseldorf, Germany), and Oxi 330 oximeter (WTW, Weilheim, Germany), respectively. Free and total chlorine were also determined *in situ* using reagent chlorine HR tablets (Fisher, Courtaboeuf, France) in a Pocket colorimeter II (Hach, Düsseldorf, Germany). One supplementary liter was sampled in each DWST at each campaign to measure biodegradable dissolved organic carbon (BDOC) and total organic carbon (TOC), as described by Joret et al. (1991).

2.2. Sample collection

The samplings were performed in duplicates in 3 different DWST, named towers TA, TB, and TC, from the metropolitan Paris area. Towers TA, TB and TC were characterized by a height of 26.1 m, 20.8 m and 57.5 m, a tank depth of 8.4 m, 8.5 m and 8.5 m and a capacity of 2200 m^3, 2850 m^3 and 2100 m^3, respectively, and were all made in concrete. The selection of DWST was based on the water residence time in the distribution network from the treatment plant. This time was estimated as 8.8–59.7 h, 15.4–47.8 h and 48.1–134.5 h for tower TA, TB and TC, respectively.

Concrete surface chemistry (pH) differs as a function of its age (Heng and Murata, 2004). However, no concrete block was made at the time of DWST construction rendering impracticable the biofilm analysis on supports displaying a surface chemistry similar to the DWST walls. Glass supports were used as a model in this work to analyze biofilm colonization of the DWST surface and were preferred over concrete because of (i) the rough surface of concrete materials rendering complex the collection of biofilm integrality, as opposed to the smooth surface of glass plates, and (ii) the difficulty to set up confocal microscopy analysis with concrete slides. Furthermore, as the attachment of microorganisms is promoted less rapidly on glass, a hydrophilic material, than on hydrophobic nonpolar surfaces, such as Teflon or other plastics (Bendinger et al., 1993; Fletcher and Loeb, 1979; Pringle and Fletcher, 1983), glass was preferred over other plastic materials. Thus, sterile borosilicate plates (10 cm × 10 cm × 1 cm) or glass microscope slides specially designed for this study were set up vertically in stainless steel protection cages (Fig. 1A) placed at the surface (50 cm under water surface), the middle (middle of the water column) or the bottom (50 cm above of the water tank floor of the water tower) of the DWST to analyze biofilm development.

The seasonal and kinetics studies were conducted in two distinct sampling campaigns. For the seasonal study, water samples were collected in an initial sampling campaign in 04/2014 (spring 2014) used to set up devices in the tower for biofilm collection 3 months later. No biofilm samples were collected in the spring 2014 sampling campaign. Water and biofilm samples were collected at each season every 3 months during 4 sampling campaigns: 07/2014 (summer 2014), 10/2014 (autumn 2014), 01/2015 (winter 2015) and 04/2015 (spring 2015). Samplings were performed at 3 different levels (surface, middle