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# MICROBIOLOGICAL DRINKING WATER QUALITY AND BIOFILM FORMATION ON A DISTRIBUTION SYSTEM: COMBINED EFFECTS OF WATER ORIGIN, RESIDENCE TIME AND MATERIALS.

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## ABSTRACT.

From september 2002 to march 2003 two different types of biofilm reactors ( polyethylene Propella™ and Water Boxes Devices™ made of polyethylene, copper and lead ) have been connected to surface water and groundwater at the beginning and at the end of the parisian distribution network. Microbiological analyses included enumeration of culturable bacteria (PCA agar), total bacteria (DAPI), metabolically active bacteria (CTC) and semi-qualitative analysis of the total bacterial population by Fluorescence In Situ Hybridation (FISH) were carried out on biofilms sampled from the two different types of biofilm reactors and waters at the inlet and outlet of each experimental systems. The FISH used method allows the detection and enumeration of total eubacteria and  $\alpha$ ,  $\beta$  and  $\gamma$  eubacteria groups. The obtained results prove that there is a significant difference between surface water and groundwater in terms of total bacteria and  $\beta$  bacteria. Effect of water residence time (WRT) in the network is also observed with an increase of metabolically active cells, eubacteria and  $\alpha$  bacteria in distributed water which is linked to free chlorine consumption. No effects of WRT or water origin was noted on biofilm density and composition while copper and lead induce a decrease of culturable bacteria in water.

## KEYWORDS.

Biofilm, FISH, microbiological drinking water quality, pipes materials, residence time, water origin.

## INTRODUCTION.

In all drinking water distribution systems, in spite of disinfectants, microbiological water quality can deteriorate due to biofilms (Paquin *et al.*, 1992). Mostly constituted of bacteria enclosed in an extracellular polymeric slime (Flemming and Wingender, 2001) which prevents the action of disinfectants (de Beer *et al.*, 1994; Lu *et al.*, 1998), biofilms in distribution networks present a lot of disadvantages: contamination of the distributed water (detachment of fixed bacteria), occurrence of tastes and odours, bio-corrosion of the pipes or disinfectant decay.

The new european regulation specifies the evaluation of water quality not anymore at the outlet of treatment plants but at the consumer's tap. So, it is important now for drinking water suppliers to have efficient tools for the quantification of biofilms, in order to detect disorders and react faster as possible if necessary.

A lot of studies about biofilm occurrence in distribution systems have been realised yet and several methods of biofilm sampling exist. More precisely, biofilm can be removed directly by scraping the colonisation surface (Schoenen, 1989; Lund and Ormerod, 1995) or collected in experimental incubators after their exposition to the water (Camper *et al.*, 1999; Chandy and Angles, 2001; Block *et al.*, 1993). In this case, the most frequently employed method consists in a removal of the biofilm using ultrasounds (Momba *et al.*, 1998; Mathieu *et al.*,

1992) after a rinsing stage of the colonised area or not. This kind of experimental incubators allows the quantification of fixed biomass with various methods: heterotrophic plate count analyses, direct count of total bacteria, ATP measurement etc.

SAGEP is the water supplier for the city of Paris (750,000 m<sup>3</sup> per day). Drinking water is supplied for 50% by groundwaters and the rest is produced in three surface waters treatment plants.

Previous experiments concerning the microbiological quality drinking water in the Paris network (Delahaye et al., 2003) proved that:

- the Paris network provides good-quality waters with bacteriological parameters dealing with the standards (HPC-PCA at 22°C and 37°C, total and thermoresistant coliforms, enterococci) and also with non-standardized parameters offering increased detection of viable or culturable bacteria (HPC-R2A and ATP),
- the Paris network is heterogeneous with at least two zones corresponding to surface waters and underground waters.

Because of these previous results and in order to compare surface and underground waters for their biofilm formation potential, two different types of biofilm reactors have been connected to surface and underground waters at the beginning and at the end of the parisian distribution system. This is a highly complex gridded network in which the average water residence time is six hours.

The aim of this study is to evaluate:

- the impact on biofilm formation depending on the water origin and the residence time in the network,
- the effect of three different materials (copper, lead and high density polyethylene) on the microbiological drinking water quality.

## MATERIALS AND METHODS.

### **Sampling strategy.**

As presented in table 1, four sampling sites have been used for this study. In each site two different experimental systems have been installed: a high density polyethylene (PE) Propella™ reactor (NANCIE) and a set of Water Boxes Devices™ (WBD, EPI company).

Table 1. Sampling sites of the study.

<b>Site</b>	<b>Characteristics</b>
G0	Groundwater, low water residence time in the network
G1	Groundwater, high water residence time in the network
S0	Treated surface water, low water residence time in the network
S1	Treated surface water, high water residence time in the network

The Propella™ reactor system has previously been described (Appenzeller et al., 2001; Crissot-Laruade et al., 1999). This type of reactor allowed independent flow speed and residence time monitorings. During the study, the water velocity was 0.2 m/s and the

residence time in the reactors was 12 hours. These hydraulic conditions have been chosen in order to obtain a maximal estimation of the biofilm formation.

The WBDs™ are made of pipes pieces and simulated parisian network pipes with a water velocity of 0.3 m/s. Each set of WBDs™ consisted of three WBDs™ made of three different materials: copper (Cu), PE and lead (Pb).

### **Water and biofilm measurements.**

The study took place from september 2002 to march 2003. From october 2002, on each sampling site, every month, one PE coupon from the Propella™ was removed. At the same time, free chlorine and temperature of the feeding water were measured and water was sampled at the inlet of the experimental system as well as at the outlet of the Propella™ reactor and of each WBD™. In order to carry on microbiological analyses, fixed biomass was detached from PE Propella™ coupons in sterile water using ultrasounds. In all waters and biofilm samples, the following analyses were realised:

- enumeration of culturable bacteria (HPC) using PCA agar (22°C, 15 days),
- total count of bacteria (TB) using DAPI staining as described by Appenzeller *et al.* (2001) while metabolically active bacteria (MAB) were enumerated with CTC only on water samples,
- semi-qualitative analysis of the total bacterial population by Fluorescence In Situ Hybridation (FISH). Based on the use of RNA-targeted Cy3 fluorescent probes, this method allowed the detection and enumeration of total eubacteria (EUB338) and alpha (ALF1b), beta (BET42a) and gamma (GAM42a) bacterial groups, where the gamma bacterial group includes potentially pathogenic microorganisms.

### **Statistical analyse.**

In order to compare non paired sets of data, ANOVAs at a confidence level of 95% were systematically computed using the R software (<http://cran.r-project.org/>) so that all significant differences presented in the text correspond to a p-value < 0.05.

Boxplot graphics were performed with the same software.

## **RESULTS AND DISCUSSION.**

### **Temperature and free chlorine.**

During the course of the study, groundwater exhibit highly constant temperatures (12.4 °C +/- 1.0) while consequent changes were observed for surface water (12.7 °C +/- 4.6). On the other hand, free chlorine consumption existed for the two waters in the network (table 2).

Table 2. Free chlorine consumption in the parisian network for undergroundwater and surface water.

	<b>Free chlorine (mg Cl / L)</b>
<b>G0</b>	0.18 +/- 0.03
<b>S0</b>	0.19 +/- 0.02
<b>G1</b>	0.08 +/- 0.03

<b>S1</b>	0.15 +/- 0.03
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This was a well known phenomenon (Zhang *et al.*, 1992; Nakache *et al.*, 1996; Abouzaid *et al.*, 1996; Kerneis *et al.*, 1995) due to free chlorine reduction by both biofilms and corrosion products (Hallam *et al.*, 2002; Lu *et al.*, 1998; Frateur *et al.*, 1999).

### Water origin and WRT effects.

There were significantly more TB / ml and beta / ml in surface water than in groundwater (figure 1).

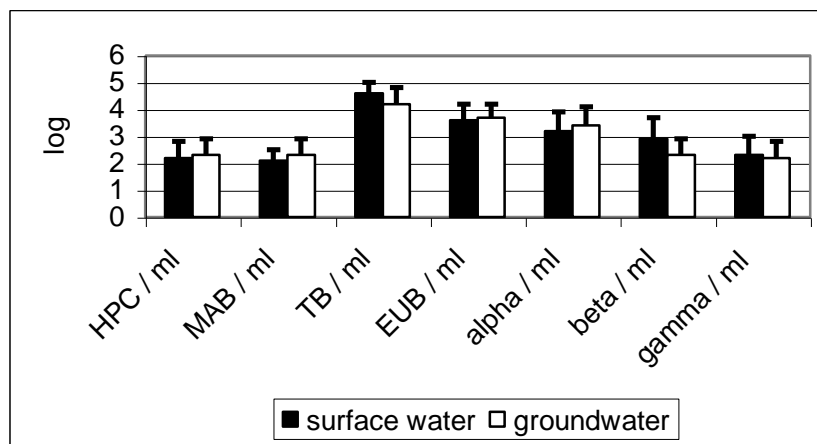


Figure 1. Microbiological parameters of waters: surface water vs groundwater.

This was not surprising and the best microbiological quality of groundwater has already been constated in the parisian network (Delahaye *et al.*, 2003) and more generally by others authors (Zacheus and Martikainen, 1995).

A WRT effect on water was also observed (figure 2) in terms of MAB / ml, EUB / ml and  $\alpha$  bacteria / ml.

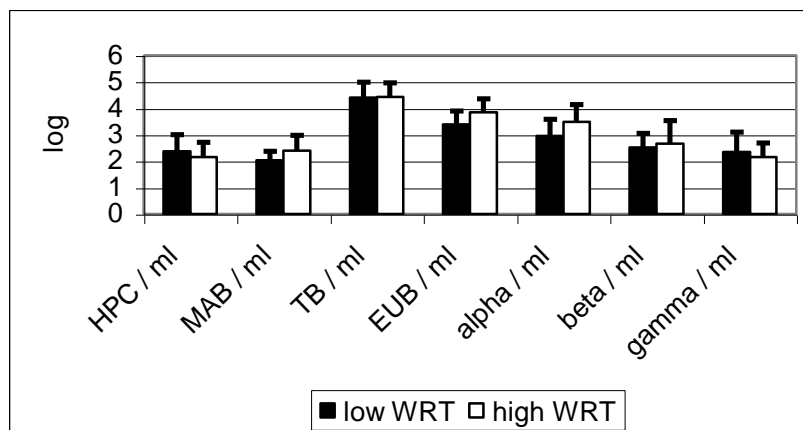


Figure 2. Microbiological parameters of waters: low WRT vs high WRT.

Slight microbiological degradation of the water during distribution has already been observed in the parisian distribution system (Delahaye *et al.*, 2003) and more generally WRT was a factor inducing water quality degradation (Van der Kooij, 1999; Nakache *et al.*, 1996; Kerneis *et al.*, 1995). This was certainly linked with free chlorine consumption (table 2) but same levels of HPC / ml were surprising because free chlorine should also decrease culturability. Even if the incubation time is 15 days, the low sensitivity of PCA-agar (Reasoner *et al.*, 1984; LeChevallier *et al.*, 1987; Delahaye *et al.*, 2003) may be regarded as responsible.

On the other hand, no effect of WRT or water origin was noted on biofilms densities and composition (figures 3 and 4).

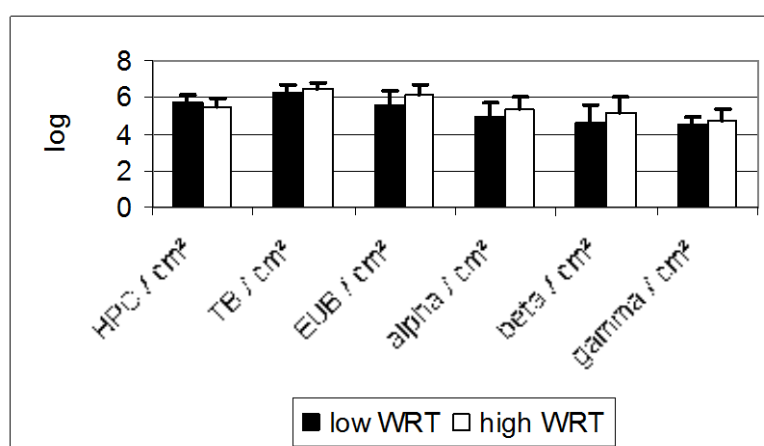


Figure 3. Microbiological parameters of biofilms: low WRT vs high WRT.

The obtained results were equivalent to those presented elsewhere for biofilms at the equilibrium state:  $10^5$  to  $10^6$  HPC/ cm<sup>2</sup> (Volk and LeChevallier,1999; Zacheus *et al.*, 2000; Van der Kooij *et al.*, 1994; Abouzaïd *et al.*,1996) and  $10^6$  to  $10^7$  TB / cm<sup>2</sup> (Zacheus *et al.*, 2000; Abouzaïd *et al.*,1996).

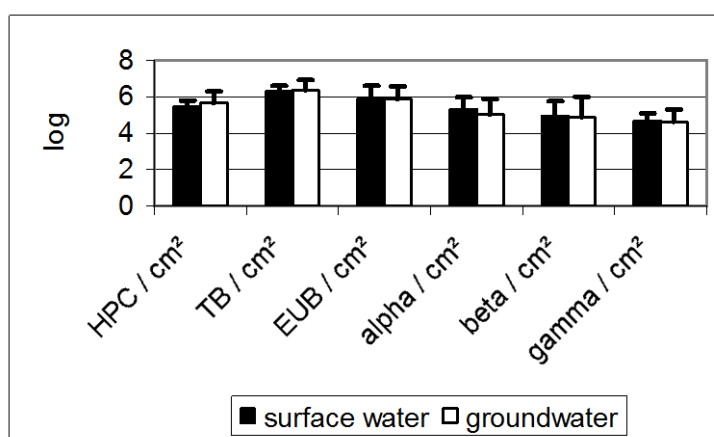


Figure 4. Microbiological parameters of biofilms: surface water vs groundwater.

**Experimental systems and materials effects.**

Figure 5 presented microbiological parameters of feeding waters, waters at the outlet of the Propella™ reactors and at the outlet of the WBD™ s (all materials considered). Whatever the considered parameter, Propella™ reactors induced an important increase of bacterial densities in water certainly due to a high residence time in the system (12 hours).

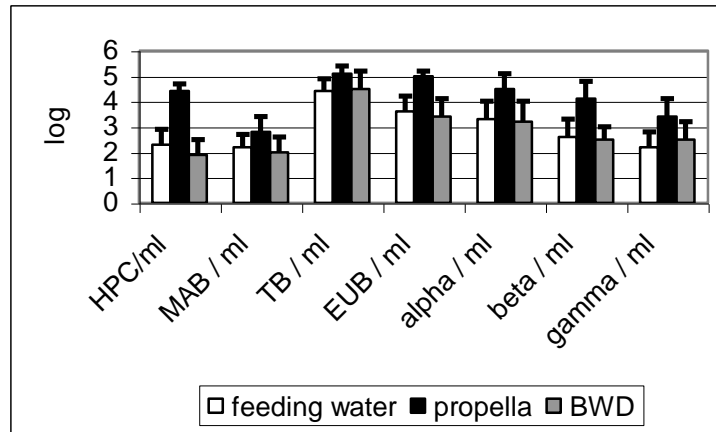


Figure 5. Microbiological parameters of waters: feeding water vs water at the outlet of Propella™ reactors vs water at the outlet of WBD™ s.

This increasing effect due to hydraulic conditions was confirmed if we compared values at the outlet of the Propella™ reactors and at the outlet of PE WBD™ (figure 6) which were made of the same material. On the other hand, all materials considered, WBD™ s induced a decrease in terms of HPC / ml (figure 5) . This was not the case for PE WBD™ with values equivalent to the feeding water (figure 7). As presented on figure 7, Cu and Pb have certainly a toxic effect on bacteria inducing a decrease in culturability while PE did not have any effect. Other studies have precedently stressed out a possible bacterial toxic effect of these metals leading to a low density of the formed biofilm (Mathieu et al., 1998).

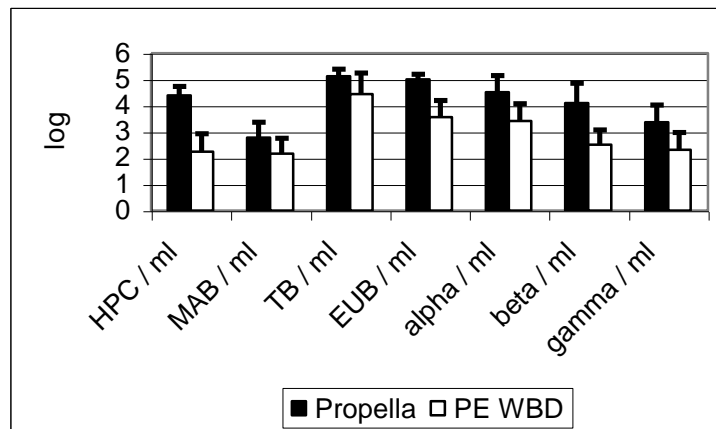


Figure 6. Microbiological parameters of waters: water at the outlet of Propella™ reactors vs water at the outlet of PE WBD™ s.

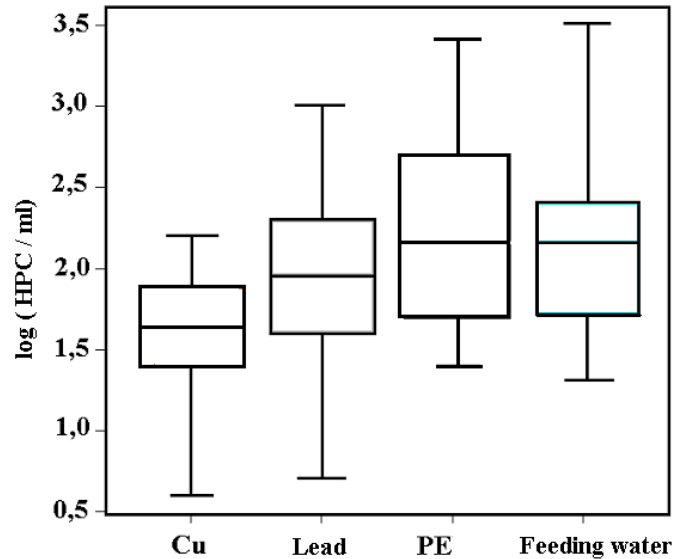


Figure 7. Microbiological parameters of waters at the outlet of WBD™ s: PE vs Cu vs Lead vs Feeding water.

## CONCLUSION.

The slight heterogeneity of the parisian distribution system was confirmed. Surface waters and groundwaters differed for their microbiological qualities and WRT has a significant effect on water quality. Nevertheless, these significant differences between waters of different origins or due to WRT do not have any effects on biofilm formation and the quantity of fixed biomass is quite the same than those presented in other studies carried on on other networks. On the other hand, the Propella™ reactor induced important changes in terms of water quality and must be considered as a relative tool for studying biofilms, like the major part of other experimental systems, except those which consisted in pipes with short residence times like the WBD™ s. Finally, materials like Pb and Cu reduced the number of culturable bacteria in water. PE did not have any effect and like Cu must be considered in order to replace Pb in private water pipes.

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