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## Abstract

Bacteriological and physico-chemical water quality parameters were measured in rainwater from 10 harvesting tanks, belonging to two types of rainwater harvesting systems (outside tanks for garden watering and underground tanks for both outdoor and indoor uses) in Île-de-France area (France). The presence of fecal coliforms, enterococci, *Pseudomonas* spp., coliforms, total flora at 22°C and 36°C and total DAPI direct counts showed a microbiological contamination in water and sediment samples for both tank types. However, the harvested rainwater quality suited some existing water quality guidelines for different non-potable purposes (bathing water or reused wastewater). To our knowledge, the presence of microorganisms in sediment compartment in rainwater harvesting tank has been studied for the first time here. Results showed that the sediment could be a reservoir of re-contamination for some microbial species, which would survive in the sediment and may be resuspended in the water column. In parallel, physical-chemical parameters (temperature, turbidity, conductivity, pH and dissolved organic carbon) of collected rainwater were found in the ranges reported in the literature. Algal blooms, principally green algae, occurred in the outside tanks during spring and summer as shown by the high chlorophyll levels (the highest chlorophyll content reached 209.8 µg/L with a water pH of 9.37). Nitrate and phosphate concentrations in water samples were low (respectively less than 5 mg/L and 0.5 mg/L) but high enough to support the development of microorganisms and especially photosynthetic microorganisms in the outside tanks. The difficulty to evaluate the microbial quality of roof-harvested rainwater due to the lack of suitable quality standards is underlined. Depending on the usage of the harvested rainwater, some treatments may be needed to get a better microbial quality and direct measurements for pathogens may be also needed to establish whether or not such harvested rainwater is safe for different types of uses.

## 1. Introduction

Harvesting and stocking rainwater for household uses becomes popular in France, not only in rural but also in urban and suburban areas. The Paris region and its neighborhood (Île-de-France area) is no exception to this trend. The Île-de-France area is the most populated region (11.6 millions) accounting for 18.5% of the population of France and the most densely populated (966 residents/km$^2$) of France. There are an increasing number of individual houses equipped with tanks to harvest rainwater. Harvested rainwater is used for garden watering, floor and car washing or other advanced uses like toilet flushing, laundry washing and some other advanced uses. But little is known about the sanitary risk it represents. Microbiological and chemical quality of roof-harvested rainwater could be impaired by animal droppings, moss, lichens, windblown dust, and particulates from urban pollution. Knowledge on quality of roof-harvested rainwater and its evolution in tanks could help to prevent potential risks associated with its different uses. However, few data concern the microbiological quality of roof-harvested rainwater in France.

Our research project aims to measure the physical-chemical and microbial quality of collected rainwater and to evaluate their evolution depending on storing type and use. Two case studies will be discussed: the outside tanks for outdoor utilization and the underground tanks for outdoor and indoor utilizations (toilets flushing, laundry washing and other advanced uses). The presence of fecal coliforms, enterococci, *Pseudomonas*, coliforms, total flora at 22°C and 36°C and total DAPI counts will be analyzed in two types of samples collected (water and sediment). In parallel, physical-chemical parameters (temperature, turbidity, conductivity, pH, dissolved organic carbon (DOC) and chlorophyll content) of collected rainwater will be monitored in order to understand the evolution mechanism of water stored in tanks.

## 2. Description of the region and categories of tank

Île-de-France area has a temperate climate and its precipitation is close to 600 mm. A total of ten individual houses were chosen to conduct all sampling campaigns: five houses in Champigny-sur-Marne (Val de Marne, France) and five houses in Chaumontel (Val d'Oise, France). These sites represented interesting model situations for evaluating rainwater quality. In Champigny-sur-Marne, the county administration installed unique model of outside tanks in 30% houses for roofs rainwater harvesting. In Chaumontel, all houses of a recent block were equipped with an underground tank. For each category of tank (outside or underground), all sampled houses uniformly endured the same weather condition during sampling period.

For outside tanks, rainwater was harvested in a four year old model of 800 litters made of polyethylene high density – PEHD for different outdoor uses. For underground tanks, two to four year old PEHD models had volumes varying from 3 000 litters to 5 000 litters and possessed a screening box at the entrance. The harvested rainwater was used for outdoor use, toilet flushing, laundry washing, bathing (in one house) and swimming pool filling (in one house). All houses possess a tiled roof which was not cleaned and chemically treated.

## 3. Materials and methods

Rainwater sampling was processed after cleaning the tap three times with ethanol. The sediment was sampled using the Sigma SD900 Potable Sampler. A plastic pipe was inserted into the tank to reach the sediment which was sucked by the pump and collected in a sterile plastic tube. For outside tanks, sampling campaigns were carried out in the period from April to October when harvested rainwater was frequently used. For underground tanks, samples were performed in the period from December to February.

The physical-chemical parameters were only performed in rainwater. Some parameters (temperature, conductivity and pH) were measured *in situ* by using the WTW Multi 340i/SET. Dissolved organic carbon concentration was evaluated using a1010 O.I.Analytical total Organic Carbon Analyzer. Nitrate and phosphate contents were estimated by the

Palintest Photometer 7000se, after sample filtration on Whatman GF/F membrane (0.7 µm pore size, 47 mm diameter). Turbidity measurement was performed by the HACH 2100p turbidimeter in the laboratory. For rainwater samples of outside tanks, the chlorophyll content was measured using Lorenzen method and performed by the JASCOFP-750 spectro-fluorometer and the PERKLIN ELMER UV/VIS Spectrometer lambda 11.

For bacterial parameters, all samples (rainwater and sediment) were collected and analysed in sterilized equipments. The samples were transported in cool isothermal boxes and processed within 24h. Fecal coliforms and coliforms, intestinal enterococci and *Pseudomonas* were analyzed by membrane filtration following the international standard methods ISO 9308-1, ISO 7899-2 and ISO 16266 respectively. Results were expressed as colony-forming units (CFU) per 100 ml of sample. When necessary, dilution was done to reach a countable number of colonies on plate. For culturable microorganisms, the standard method ISO 6222 was performed by counting the colonies formed in nutrient agar medium culture after aerobic incubation at 36°C and 22°C. Results were expressed as CFU per ml of sample. Total cells were directly counted on black polycarbonate filters (0.2 µm pore size) by fluorescent microscopy after staining with 4′, 6-diamidino-2-phenylindole (DAPI). All parameters were evaluated over a total of at least five houses. All results were statistically analysed using PAST software 1.88 (Hammer *et al.*, 2001).

## 4. Results

### 4.1. Physical–chemical parameters measurements

Results for physical–chemical parameters of rainwater from outside and underground tanks showed high variability between houses and tanks (table 1). The temperature of rainwater stocked in underground during winter sampling campaigns was around 8°C. The average pH value measured in rainwater of outside tanks was at a base level, 8.67 (7.64 minimum and 9.38 maximum), while the average pH of rainwater from underground tanks was lightly acidic, 6.21 (5.49-6.8). The nitrate content was within the same range in both types of tank. The average amount of chlorophyll content in rainwater from outside tanks was 57.4 µg/L (0.3 minimum and 209 maximum).

Table 1: Average levels of physical-chemical parameters of rainwater measured from outside and underground tanks (*n* corresponds to the number of samples; numbers in parenthesis correspond to minimum and maximum values)

| Physical-chemical parameters | Outside tank | Underground tank |
|---|---|---|
| | n = 6 - 9 | n = 5 - 6 |
| pH | 8.67 (7.64-9.38) | 6.21 (5.49-6.8) |
| Conductivity (µS/cm) | 34.29 (10-80) | 50.2 (22-88) |
| Turbidity (NTU) | 2.96 (0.95-6.20) | 1.31 (1.06-1.69) |
| DOC (mg/L) | 1.93 (0.5-4.55) | 1.09 (0.73-1.61) |
| Nitrate (mg/L) | 2.29 (1.29-3.90) | 2.67 (1.26-4) |
| Phosphate (mg/L) | 0.23 (0.12-0.34) | 0.068 (0.03-0.12) |

### 4.2. Microbial analysis

Results for indicator of faecal contamination and total DAPI counts of rainwater and sediment from two types of tank were presented in table 2. For outside tanks, the average means for each indicator bacterial group (total coliforms, fecal coliforms and enterococci) were respectively 303, 2 and 2 per 100 ml for rainwater and 24004, 67 and 0 per 100 mL for sediment. The presence of *Pseudomona*s spp. was high in sediment (table 2). For underground tanks, the rainwater was characterized by an average mean level of 373 cfu/100 mL for total coliforms, 31 cfu/100 mL for fecal coliforms and 109 cfu/100 mL for enterococci. The level was high for sediment samples: 19041, 667 and 61 cfu/100 mL for total coliforms, fecal coliforms and enterococci, respectively. The presence of *Pseudomonas* was very important in both rainwater and sediment. The total DAPI count was at the same range of rainwater sampled in both types of tanks. Overall the concentration of microbes was generally higher in sediment than in rainwater whether outside tanks or underground tanks. We found a difference in the level of total coliforms, fecal coliforms, culturable microorganisms at 22°C and culturable microorganisms at 36°C between rainwater from outside tanks and from underground tanks.

Table 2: Average levels of faecal indicators and total DAPI counts of rainwater and sediment measured from outside and underground tanks (n corresponds to the number of samples; numbers in parenthesis correspond to minimum and maximum values).

| Outside tank (n = 5) | Water | Sediment |
|---|---|---|
| Coliforms (cfu/100mL) | 303 (0-1700) | 24004 (0-144000) |
| Fecal coliforms (cfu/100mL) | 2 (0-7) | 67 (0-200) |
| Enterococci (cfu/100mL) | 2 (0-10) | 0 |
| Total flora at 22 °C (cfu/1mL) | 1280 (420-2330) | 327500 (23500-888000) |
| Total flora at 36 °C (cfu/1mL) | 1528 (170-2880) | 32167 (29000-36500) |
| *Pseudomonas* spp. (cfu/100m | 87 (3-240) | 3233 (0-10000) |
| Total DAPI count (x$10^6$ cells/m | 1.4 (0.6-2.3) | 104 (36.5-234) |
| **Underground tank (n = 5)** | **Water** | **Sediment** |
| Coliforms (cfu/100mL) | 373 (75-875) | 19041 (3000-54833) |
| Fecal coliforms (cfu/100mL) | 31 (3-127) | 667 (0-1217) |
| Enterococci (cfu/100mL) | 109 (0-282) | 61 (0-167) |
| Total flora at 22 °C (cfu/1mL) | 374 (21-1291) | 102693 ( 13135-436364) |
| Total flora at 36 °C (cfu/1mL) | 201 (41-467) | 187025 (1 2727-860000) |
| *Pseudomonas* spp.(cfu/100mL | 1618 (11-7567) | 81210 (4636-324324) |
| Total DAPI count (x$10^6$ cells/m | 1.6 (1.2-2.1) | 53.8 (6.8-103.0) |

## 5. Discussion and conclusion

The importance of individual houses in Île-de-France (about 30%) makes this region the greatest potential for development of installations of rainwater collection systems. In France, harvested rainwater is used not only for garden watering and floor cleaning but also for toilet flushing, laundry washing and other advanced uses. In this study, harvested rainwater of all our sampling sites is not used for drinking purpose. Since there is no standard for the microbiological quality applied for harvested rainwater, the comparisons of rainwater quality between studies become difficult. We thus compare harvested rainwater quality to some existing microbiological water quality levels required in France and Europe for potable and non-potable uses and some examples of rainwater quality for toilet flushing (in Canada, Japan, Germany, United Kingdom and United Stated of America) (table 3).

The high level of pH around 8.67 of rainwater harvested from outside tanks could be due to the algal development throughout the photosynthesis. This phenomenon was confirmed by the presence of algae and high content of chlorophyll in rainwater. In one outside tank where pH was 9.37 and the chlorophyll content was 209.8 µg/L, we observed the green coloration of stocked rainwater. This chlorophyll content was high in comparison to the one found in water from the trophic lake (2-90 µg/L; Thiemann and Kaufmann, 2000). The algal development could be promoted by the elevated level of temperature and light because all outside tanks were installed along the house wall and without protection against the sun. Algal blooms occurred in outside tanks during the spring and summer as shown by the chlorophyll levels. The cyanobacteria were always under represented; mainly green algae dominated the bloom (data not shown).

Table 3: Summary of microbiological water quality requires for potable and non-potable use

| Indicators | Drinking water<br>French minister's order (Jan/11/2007) | Bathing water<br>European directive (CE/Jul/2006) | Reuse water<br>French minister's order (Aug/02/2010) | Examples of international microbiological water quality criteria for toilet flushing |
|---|---|---|---|---|
| Escherichia coli | 0 | 900* | <250[d] | O[a*] (US)<br>O[b*] (UK)<br>10[b] (Japan)<br>100[b] (Germany)<br>200[c] (Canada) |
| Intestinal enterococci | 0 | 330* | | |
| Total coliforms | 0 | | | 240[a*] (US)<br>500[b] (Germany)<br>10[b] (Japan) |

* 90th percentile evaluation; [a] U-S Environmental Protection Agency, 2004; [b] Birks et al., 2004;
[c] Canadian guidelines, 2007; [d] good quality level of reuse water for irrigation

The average pH of rainwater from underground tanks was slightly higher than the level of ambient rainwater (5.9) measured by Mendez *et al.* (2011) showing that tile-roof runoff and stagnation in PEHD tanks only slightly changed the pH. Conductivity remained at low level in both types of tank which showed that harvested rainwater was not very charged by dissolved salts or other impurities from the tile-roof, gutters and pipes. Turbidity seemed more acceptable in rainwater harvested from underground tanks compared to outside tanks, being below the values were reported by other authors (10-56 NTU by Yaziz *et al.*, 1989 and 5-35 NTU Mendez *et al.*, 2011). DOC was found in low levels in comparison with literature results which varied from 2 to 56 mg/L (Mendez *et al.* 2011, Yaziz *et al.* 1989). The nitrate and phosphate contents were also low but this level of nitrate and phosphate were high enough to support the development of microorganisms and especially photosynthetic organisms in the outside tanks.

The average of each indicator bacterial group (total coliforms, fecal coliforms and enterococci) measured in rainwater in both types of tank was found in ranges reported in previous studies (Savill *et al.*, 2001; Simmons *et al.*, 2001; Vasudevan *et al.*, 2001; Evans *et al.*, 2006; Evans *et al.*, 2008; Vialle *et al.*, 2010; Mendez *et al.*, 2011). These levels all exceeded the mean guideline for drinking water purpose but it was below the level of guidelines for bathing water and reuse water (table 3). The average of total flora at 22°C and 36°C was within the levels found by Rosillon *et al.* (2007) an Amin and Han (2009) but remained low in comparison with the levels found by Simmons *et al.* (2001), Birks *et al.* (2004) and de Gouvello and Noeuvéglise (2007), demonstrating the importance of other external parameters on the evolution of water composition (i.e. rain quality, geographic area, tank composition, intrinsic bacterial flora…). The presence of *Pseudomonas* observed in our study remained lower than in previous reports (Uba and Aghogho, 2000; Evans *et al.*, 2008) even for sediment samples. The levels of total coliforms, fecal coliforms, enterococci and total flora at 22°C and 36°C found different between outside tanks and underground tanks could indicate the role of environmental conditions in the survival of these microorganisms however the samples were collected at different seasons, which made difficult to compare the two kinds of tanks. The level of faecal indicators remaining lower in outside tanks than underground tanks supposed that this could be reduced in such condition. In our case, we thought the variation of temperature, high pH and the presence of microalgae could play an important role in reducing these microorganisms. However, the total flora at 22°C and 36°C remaining lower in underground tanks than in outside tanks could be due to stable and low temperature in inside of this last one. Although the levels of total flora at 22°C and 36°C were changed, total DAPI counts were comparable in both types of tanks. This indicated that the different environmental conditions in two types of tanks changed the microbial composition in stocked rainwater. For the first time to our knowledge, the presence of microorganisms in sediment compartment in rainwater harvesting tank has been studied. We observed a correlation between fecal coliforms and *Pseudomonas* spp. densities in sediments and water supported the hypothesis that the sediment compartment could be a reservoir of re-contamination for some microbial species which would survive in the sediment and may be resuspended in the water column. However, we need more data analysis to confirm this statement.

Statistical comparison between the physico-chemical and bacterial parameters did not show significant correlation ship between nitrate content and bacterial parameters (data not shown). No solid conclusion could be made if the higher nitrate content is correlated with higher levels of some bacterial parameters. Further analysis with more samples is needed in order to establish a better understanding of the correlation between the parameters in rainwater and in sediment and between the physical-chemical parameters and bacterial parameters in rainwater.

In our study, the harvested rainwater was used only for non-drinking purpose. In general, the microbiological analysis of harvested rainwater seemed in accordance with some existing guidelines for non-potable uses. Faecal indicators presence could indicate animal dropping contamination; however these indicators could not indicate the presence of human pathogens and especially environmental waterborne pathogens such as legionella or non-tuberculous mycobacteria and parasitic protozoa. So, it could be inadequate to use the indicators of faecal contamination for roof-harvested rainwater quality which is not probably polluted by human waste contamination. Moreover, it is very difficult to confirm whether such roof-harvested rainwater has a good quality or not if we could simply use some existed standards for other types of water such as bathing or reused waters. This showed that there is a lack of a suitable standard for roof-harvested rainwater quality corresponding to different uses. We also need a harmonized standard for European community for the roof-harvested rainwater quality in function of its uses. The chemical analysis and direct identification of pathogens may be warranted to establish whether or not such harvested rainwater is safe for different uses.

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