

## Water reuse for urban landscape irrigation: aspersion and health related regulations

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### ABSTRACT

The Mediterranean seaside resort of Le Grau du Roi includes 40 hectares of landscaped areas spray irrigated with river water supplied through a separate network. Wastewater collected from several municipalities is treated in an activated sludge wastewater treatment plant (WWTP) and polished in waste stabilization ponds (WSPs). Planned substitution of treated wastewater for river water is hindered by spray irrigation prohibition within a 100 m distance from houses and recreational areas. WWTP and WSP effluents were monitored for pathogens with a particular attention to *Legionella* in Spring and Summer 2006. Helminth eggs, salmonellae and enteroviruses were never detected neither in WWTP effluent nor in the ponds. *Legionella* spp content was slightly higher or of the order of magnitude of river water contents. Regarding *Legionella pneumophila* contents, WSP effluent did not significantly differ from the river water. *E. coli* and enterococci contents in WSP effluents complied with the "excellent quality" criteria of the European Directive for coastal bathing waters. Therefore, substituting WSP effluents to river water is unlikely to alter health risks related to spray irrigation and, in this case, the buffer zone required by the French water reuse guidelines appears being short of support.

**Key words** | landscape irrigation, *Legionella*, pathogens, regulations, water reuse

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### INTRODUCTION

In those European and Mediterranean regions which rely on tourism industry for their development, many municipalities are reusing or contemplating possible reuse of municipal wastewater to irrigate landscaped areas and golf courses. Most of these areas are mainly spray irrigated lawns located close to residential areas, lanes and roadways. Health related reuse guidelines and regulations are not yet well established in European and Mediterranean regions; correlatively, the microbial quality required for urban landscape irrigation remains a matter of debate, thus slowing down the approval of several projects.

According to US EPA guidelines, wastewater should be successively secondary treated, filtered and disinfected such that no faecal coliform could be detected in 100 mL and no level of viable pathogens measured in the reclaimed water

(*Environmental* 2004). In Australian guidelines, the microbial target is *E. coli* < 1 per 100 mL (*NRMMC-EPHC* 2006). As high reclaimed water quality is anticipated, no setback distance between irrigation sites and residential areas and roadways is suggested. French guidelines illustrate a different approach. They allow using A quality water (A quality is defined by the following criteria:  $\leq 10^5$  faecal coliforms per 100 mL and  $\leq 1$  intestinal nematode per L) but aspersion is forbidden during opening hours; low throw sprinklers should be used and a 100 m setback distance from houses, sport and recreational areas should be enforced (*CSHPF* 1991). In most Mediterranean countries, no setback distances are set, even when green spaces are spray irrigated; regulations and draft guidelines focus more on a *E. coli* content criterion:  $\leq 10$  CFU/100 mL in Italy,  $\leq 50$  CFU/100 mL in Cyprus,  $\leq 100$  CFU/100 mL in the

Greek draft and  $\leq 200$  CFU/100 mL in the regulations of Andalusia and Balearic islands and in the Spanish draft.

Urban irrigation guidelines aim at addressing health risks related to human contact with the irrigated areas - mainly lawns - and to the spreading and subsequent absorption by dwellers and passers-by of pathogens borne by spray and aerosols. Specific risks resulting from spray irrigation were the main reason for including setback distances in the French guidelines. However, the special attention given to these risks is based on limited scientific evidence. Very few data have been published on pathogen or faecal indicator transport by spray and aerosols resulting from aspersion with wastewater or reclaimed water. Devaux (2001) searched for the presence of faecal indicators in spray and aerosols at 10–150 m distance from a gun-type sprinkler irrigating corn in the main French water reuse project of Clermont-Ferrand. No bacterial indicator of faecal contamination was observed with irrigation water concentrations of less than  $10^3$  thermotolerant coliforms or streptococci per 100 mL and exposure time of 20 minutes. The few epidemiological and serological researches that have been conducted, most of them in Israel and the USA, are summarized in WHO (2006). There has not been any documented disease outbreak resulting from spray irrigation with water of *E. coli*  $\leq 10^3$  CFU/100 mL.

Otherwise outbreaks of the Legionnaires' disease, particularly in the vicinity of cooling water towers, have provided a sufficient reason to put the risks related to the spreading of pathogens by spray and aerosols on top of the agenda of health authorities in several countries. This has led to including a specific criterion in a Spanish draft: *Legionellae* spp  $\leq 10^3$  CFU/100 mL.

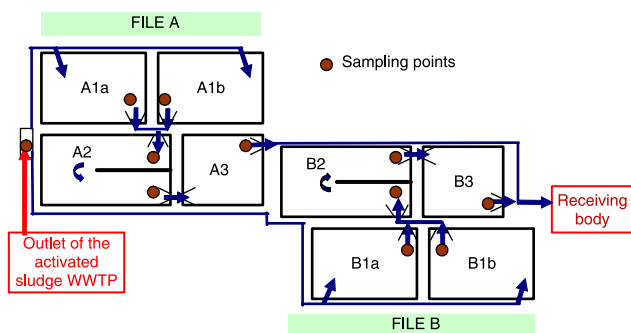
Le Grau du Roi, a seaside resort in Southern France, includes more than 40 hectares of landscaped areas; most of these areas are spray irrigated with raw Rhône River water supplied through a separate network dedicated to irrigation. Local authorities contemplate substituting reclaimed wastewater for the Rhône water currently used for landscape irrigation. This water reuse plan is driven by economic reasons and is eased by the existence of the separate network, a wastewater treatment plant (WWTP) flow rate that more than matches the irrigation needs, the high quality of WWTP effluents and the existence of waste stabilisation ponds (WSPs) which provide both an additional treatment and a storage facility for the treated water. However, most of the landscaped

areas of Le Grau du Roi are spray irrigated lawns close to buildings and roads. Given the current French guidelines, aspersion of these green spaces with reclaimed water should be prohibited. In order to help health authorities considering that this measure may, in some circumstances, be over restrictive, the microbial quality of the effluents of Le Grau du Roi WWTP and WSPs was monitored in Spring and Summer 2006. The objective was to determine whether these effluents contain pathogens and whether there is a significant difference in the microbial quality between these effluents and the currently used Rhône water. A particular attention was paid to *Legionellae*.

## MATERIALS AND METHODS

Sewage treated by Le Grau du Roi wastewater treatment facility is mainly domestic and collected from the municipalities of Le Grau du Roi and Aigues Mortes. Applied loads are characterized by large seasonal variations due to the fluctuation of tourist population. The effluent flow rate of the biological system varies from 4,000 m<sup>3</sup> per day in autumn and early spring to 10,000–12,000 m<sup>3</sup> d<sup>-1</sup> in July and August. The biological WWTP started operating in 1997. Its treatment capacity is 24,000 m<sup>3</sup> d<sup>-1</sup>. The plant was designed to remove suspended solids (SS), organic matter and also nitrogen and phosphorus. Two identical trains allow coping with seasonal variations. Mean WWTP effluent characteristics were respectively, 9.4, 3.3, 32, 6.6 and 0.8 mgL<sup>-1</sup> for SS, BOD<sub>5</sub>, COD, total N and total P.

The WSP plant was constructed in 1966 and was the only wastewater treatment facility of Le Grau du Roi seaside resort until 1997. Nowadays, it provides a tertiary treatment. Ponds total area is 21 hectares. There are two parallel pond trains (Figure 1). Effluents of the two trains mix beyond the outlet of the tertiary ponds and feed a brackish lagoon. Stored water volume varies between 23,600 and 48,700 m<sup>3</sup> from one pond to another. Besides, biological WWTP effluent is not equally distributed between the two pond trains A and B and between the two primary ponds of each train. Thus, water residence times vary from one pond to another and with seasonal flow rate variations. As a consequence, the total WSP residence time varies from 19 days in summer in file B1b-B2-B3 to 186 days in winter in file A1b-A2-A3. SS contents in WSP effluent



**Figure 1** | Lay out of Le Grau du Roi WSPs and location of the sampling points.

varied between around  $40 \text{ mgL}^{-1}$  in early spring and  $8 \text{ mgL}^{-1}$  in August and July,  $\text{BOD}_5$  between  $10.5 \text{ mgL}^{-1}$  in spring and  $7 \text{ mgL}^{-1}$  in summer, COD between a mean spring value of  $65 \text{ mgL}^{-1}$  and  $44 \text{ mgL}^{-1}$  in summer. Most  $\text{N-NH}_4$  concentrations were  $\leq 0.2 \text{ mgL}^{-1}$  and  $\text{N-NH}_4$  never exceeded  $0.5 \text{ mgL}^{-1}$ .  $\text{N-NO}_x$  was always less than  $0.5 \text{ mgL}^{-1}$ . Total P content remained around  $0.4 \text{ mgL}^{-1}$ .

Water was sampled at the outlet of the biological plant and at the outlets of two primary ponds, A1a and B1b, and of the two tertiary ponds, A3 and B3 (Figure 1). Additional sampling was made fortnightly at the outlet of every pond for *Escherichia coli* and Enterococci analysis. Microbial quality of the Rhône river water (quoted BRL), which is currently used for landscape irrigation, was also monitored.

Microorganisms were analysed according to the following methods:

- *E. coli*: microplate method (plates Quanti-tray 0–2000 CFU/mL IDEXX Colibert) and NF EN ISO 9308-3 French standard,
- Enterococci: microplate method (plates Quanti-tray 0–2000 CFU/mL IDEXX Enterolert),
- *Salmonella* spp: ISO 6340,
- Helminth eggs: a modified EPA method,
- Enteroviruses: XPT 90–451,
- *Legionella* spp and *Legionella pneumophila*: quantitative PCR method (XPT 90 471).

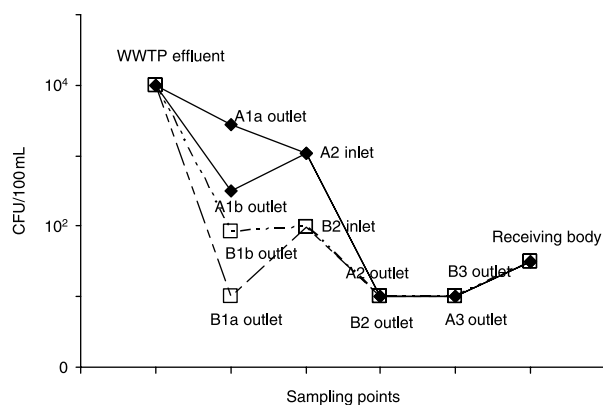
## RESULTS

### *Escherichia coli* and streptococci

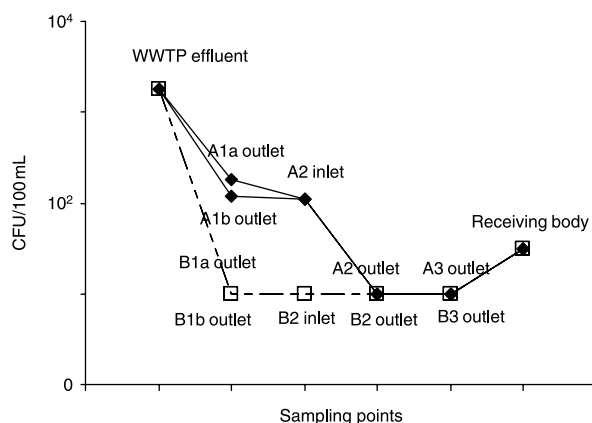
At the outlet of the biological plant, *E. coli* contents were high, between  $10^4$  and  $10^5$  CFU/100 mL. Then, from the

outlet of the primary ponds to the outlet of the whole WSP system, *E. coli* contents decreased and ranged between 5000 and  $<10$  CFU/100 mL (Figure 2). In the effluent of the WSP system, most values were below the detection limit (10 CFU/100 mL); they never exceeded 100 CFU/100 mL, which manifested a quality higher than recommended in the European Directive applying to bathing waters:  $E. coli < 500 \text{ CFU/100 mL}$  (EC 2006). The rapid bettering of water quality across the primary ponds, i.e. after a relatively short residence time was a very promising result, for abstractions for irrigation will result in a decrease of residence times. *E. coli* content in Rhône water was always below the detection limit.

Variations of enterococci contents looked much like those of *E. coli*. Indeed, most of the time, the removal ranged between 2 and 3 log units in the primary ponds (Figure 3). Contents were lower than 100 CFU/100 mL at the outlet of



**Figure 2** | *E. coli* contents on July 17th, 2006. The detection limit is 10 CFU/100 mL.



**Figure 3** | Enterococci contents on July 17th, 2006. The detection limit is 10 CFU/100 mL.

the WSPs and lower than the standard recommended by the European Directive for bathing waters: enterococci <200/100 mL. This criterion was already met at the outlet of the primary ponds, at the exception of pond A1a on August 1st.

### Helminths

Searched parasites were the followings: ascaris, trichurides, toxocara, taenia and hymenopolis. No parasite egg was found in 5 litres of water, either in the biological plant effluent or in the ponds or in the Rhône water. Because of the systematic absence of helminth eggs in April and May, they were not analyzed in June and August, but analysed again in September as a control. The absence of the searched parasites may be explained either by the absence of helminth eggs in the sewage or by the efficacy of the treatment performed in the biological WWTP. Schwarzbrod *et al.* (1989) found only 8 eggs per litre in raw wastewater in an investigation made in Eastern France, which points to the generally low helminth contamination in the country.

### Salmonella spp

In April, May, August and September (salmonellae were not analyzed in June), no *Salmonella* spp was detected in 1 litre of water, wherever the sample was taken, in the Rhône water supply, in the ponds or at the outlet of the biological plant. The literature reports that *Salmonella* spp contents in raw wastewater vary from a few tens to several hundred thousands per litre (Crook 1998; Yates & Gerba 1998; Cooper & Olivieri 1998; Jimenez 2003; WHO 2006). In Europe, contents are in the lowest part of the range most of the time. The literature reports also that the removal of *Salmonella* spp is of the order of 2 log units in conventional biological treatment plants; as the biological plant of Le Grau du Roi has been designed to achieve higher performance, it is likely to remove more than 2 log units of *Salmonella* spp. Therefore, the absence of *Salmonella* spp in the effluent of the biological plant cannot be considered surprising.

### Enteroviruses

Enteroviruses are responsible for gastroenteritis, eye infections and respiratory disease; therefore, searching for enteroviruses is consistent with the aim of assessing health

risks related to contaminated spray and aerosols. No enterovirus was detected in 10 litres in April, May, June, August and September either in the Rhône water or in the ponds or at the outlet of the biological plant. The absence of enterovirus in the effluent of the biological plant was not that expected. Indeed, the search for enteroviruses in raw wastewater is always positive, even if reported concentrations vary within a large range with, for instance, 17,000 PFU/L by Pearson *et al.* (1995), from several thousands to several millions per litre in Jimenez (2003) or Dahling *et al.* (1989). Virus removal in conventional biological plants is estimated to be around 2 log units (Jimenez 2003). In France, Gantzer *et al.* (1998) have found contents between 1.5 and 22.5 NPPUC (most probable number of cytopathogenous units) per litre at the outlet of a conventional biological plant but they also have observed the absence of enterovirus in 20 litres of the effluent of a plant similar to the Grau du Roi WWTP. Therefore, it appears that a biological treatment which eliminates most of the nitrogen and phosphorus is also able to efficiently remove enteroviruses.

### Legionella

*Legionella* develop in aqueous aerobic media at temperatures between 25 and 42°C. They use to colonize other organisms, such as amoebae or ciliates. Most often reported contamination sources are cooling towers and systems supplying hot or just warm water and producing aerosols. Risks related to *Legionella* must be considered for the following reasons:

- in Summer, water temperature in the WSPs exceeds 20°C and reaches up to 30°C;
- protozoa are a component of the ponds ecosystem which may participate in the survival of *Legionella*;
- aspersion of landscaped areas produces spray and aerosols.

It is admitted that among *Legionellae* spp, *Legionella pneumophila* is responsible for 75 to 90% of Legionnaire's diseases detected in France. This is the reason why a particular attention was given to this species.

The conventional enumeration of *Legionella* is a solid selective medium plating method (French NF T90-431 standard). In this method, only bacteria that can be

cultivated are taken into account and the detection level is around 50 CFU/L and the quantification threshold 250 CFU/L. A culturing medium used for *Legionellae* spp enumeration favours the development of *L. pneumophila*. The PCR (*Polymerase Chain Reaction*) method (standard XP T 90471) takes into account cultivable and non cultivable viable bacteria and those hosted in protozoa; its sensitivity does not depend on the species. Results are expressed as genome units per litre (GU/L). Detection and quantification levels are respectively 160 and 960 GU/L. As shown by Yanez *et al.* (2005), Yaradou *et al.* (2007) and Medema *et al.* (2004), detection of *L. pneumophila* by culture is always associated to its detection by PCR while PCR detection does not always go with detection by culture. When *L. pneumophila* is not detected by PCR, the probability of not detection by culture is 0.98 for warm household circuits and 0.97 for cooling towers (Yaradou *et al.* 2007).

In French hospitals, alert threshold related to warm water supply is *L. pneumophila*  $\geq 1,000$  CFU/L. For water cooling towers, alert threshold is set at *Legionella* spp  $\geq 1,000$  CFU/L which, given the characteristics of the NF T90-431 culturing medium, is not that different from *L. pneumophila*  $\geq 1000$  CFU/L; the call for action threshold is a concentration 100 times higher.

*Legionella* spp content was between  $10^6$  and  $3.10^7$  GU/L at the outlet of the WWTP, between  $2.10^5$  and  $7.10^6$  GU/L in the WSP effluents and between  $10^5$  and  $10^6$  GU/L in Rhône water. *Legionella* spp content was as high at the outlet of tertiary ponds as at the outlet of the primary ones. *Legionella* spp contents in the Rhône water – as enumerated by the PCR method – were lower than counts in wastewater of just one order of magnitude. These results are consistent with concentrations observed in natural waters.

Indeed, Garrelly *et al.* (2006), analyzed wastewater samples at the inlet and outlet of several WWTPs as well as river and aquifer waters in a Southern France watershed using both plate count (NF T90-431) and PCR methods. *Legionella* spp PCR enumeration values were between  $9.10^6$  and  $6.10^4$  GU/L. The same study showed that the presence of *Legionella* spp, with mean values of  $3.2.10^6$  GU/L for wastewaters and  $3.2.10^5$  GU/L for natural waters and a potable supply, did not go, one exception apart, with the presence of *L. pneumophila* even when searched by the PCR method.

*L. pneumophila* abundance is much more relevant for public health protection issues. Contrary to what has been observed with *Legionella* spp, *L. pneumophila* concentrations were quite low (Table 1).

Apart one exception (Rhône water, April 4th), *L. pneumophila* was not detected by the PCR method or was detected but at contents too low for quantification. *L. pneumophila* was detected but at not quantifiable contents at the outlet of the biological WWTP. This means that, even if a quantitative relationship between the genome unit counts and the plate enumerations is not well established, concentration in cultivable *L. pneumophila* was likely to be lower than the above mentioned alert threshold.

*L. pneumophila* was also detected, though at non quantifiable contents, at the outlet of primary ponds in May (pond A1a), June (ponds A1a and B1b) and August (pond A1a). Non quantifiable content of *L. pneumophila* was also noticed in the effluent of the WSP system in April. *L. pneumophila*, could not be detected in all the other samples of ponds water from May to September. *L. pneumophila* was either non detectable in the ponds or, when detectable, at a content too low for quantification and likely to be lower than the hospital alert threshold.

**Table 1** | Analysis of *L. pneumophila* by quantitative PCR method

	WWTP eff	A1a outlet	B1b outlet	A3 outlet	B3 outlet	Rhône water
April 3–4	P-NQ	ND	ND	P-NQ	P-NQ	5100 UG/L
May 9–10	P-NQ	P-NQ	ND	ND	ND	ND
June 20–21	P-NQ	P-NQ	P-NQ	ND	ND	ND
August 1–2	P-NQ	P-NQ	ND	ND	ND	ND
Sept. 5–6	P-NQ	ND	ND	ND	ND	P-NQ

P-NQ: *L. pneumophila* was detected but non quantifiable. ND: *L. pneumophila* was not detectable.



*L. pneumophila* was not detected in May, June and August in the Rhône water presently used to irrigate the landscaped areas of the resort. However, it was detected at a content high enough for quantification by PCR in April and at a non quantifiable content in September.

## CONCLUSION

Faecal indicator bacteria were efficiently removed from the ponds water. Effluents of the waste stabilization pond system complied with the “excellent quality” criteria defined by the European Directive 2006/7/EC (EC 2006) for coastal bathing waters. Among the pathogens that have been searched, helminth eggs, salmonellae and enteroviruses were never detected neither at the outlet of the WWTP nor in the ponds. Therefore, it can be concluded that the health risks related to human contact with lawns that would be irrigated with Le Grau du Roi WSP effluents are acceptable.

*L. pneumophila* was either non detected in ponds water or detected but at concentrations very likely to be lower than the hospital alert threshold, which means that the related infection risks encountered if spraying lawns with Le Grau du Roi WWTP and WSP effluents are not higher than those considered acceptable in hospitals housing patients with immunity deficiencies. *Legionella* spp content in ponds has been found to be slightly higher or of the order of magnitude of contents often observed in river water. Though more sensitive than the plate count method, PCR enumeration did not allow making a significant difference between *L. pneumophila* contents in ponds water and Rhône water.

Regarding all the pathogens analysed, ponds water did not differ significantly from the Rhône water. Therefore, substituting waste stabilization pond effluents to the Rhône River water is unlikely to alter health risks related to spray irrigation. In this particular situation, the buffer zone required by the French guidelines seems to be short of rational support. This conclusion does not preclude the implementation of measures that might be taken in order to prevent the development of microorganisms, including *Legionella*, in storage and distribution facilities.

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