

# Microbiological water quality improvement from reclaimed water discharges into Ridaura River (Costa Brava, Girona, Spain)

A. Costan\*, M. Montemayor\*, F. Lucena\*, J. Jofre\*, J. Muñoz\*\*, S. Quintana\*\* and L. Sala\*\*\*

\*Dept. Microbiologia, Facultat de Biologia, Universitat de Barcelona. (E-mail: [acostanlo@ub.edu](mailto:acostanlo@ub.edu); [mmontemayor@ub.edu](mailto:mmontemayor@ub.edu); [flucena@ub.edu](mailto:flucena@ub.edu); [jjofre@ub.edu](mailto:jjofre@ub.edu))

\*\*Empresa Mixta d'Aigües de la Costa Brava, SA. E-mail: ([jmunoz@aiguescb.com](mailto:jmunoz@aiguescb.com); [squintana@aiguescb.com](mailto:squintana@aiguescb.com))

\*\*\*Consorci de la Costa Brava. (E-mail: [lsala@ccbgi.org](mailto:lsala@ccbgi.org))

**Abstract** The Ridaura River is a temporary Mediterranean coastal stream that receives flows from the Castell-Platja d'Aro WWTP. These flows include both secondary effluent that submarine outfall cannot discharge into the sea, due to its limited dimension, and also reclaimed water surplus from the reclamation facility located near to the WWTP. As a result, the microbiological quality of this river downstream from the WWTP is affected mostly by: i) the presence or absence of natural flows; and ii) the discharges of secondary effluent and reclaimed water. In order to quantify these effects, two sampling strategies were adopted during 2004 and 2005: random, regular sampling; and a two-week intensive sampling program. The microorganisms tested included fecal coliforms, clostridia spores, bacteriophages, enteroviruses and *Cryptosporidium* spp. oocysts. The results provide information on the microbiological quality of secondary effluent and reclaimed water, the quality changes in the river as a result of secondary effluent discharges, and the improvements that could be achieved by expanding the treatment capacity of the water reclamation facility or the outfall dimensions.

**Keywords:** microbiological quality; temporary stream; wastewater reclamation

## INTRODUCTION

In recent decades, Ridaura's basin has been influenced by tourist and town-planning activities along the Costa Brava (Girona, Spain). Until 1993, Ridaura's river aquifer has been the only available source of water for all activities in the valley (including the urban water supply and the irrigation of crops and golf courses). Before this date, the Ridaura River and its aquifer have suffered serious disturbances, including contamination, raw wastewater flows (until 1983, when the wastewater treatment plant –WWTP- was set in operation) and marine intrusion. These factors, as well as the low rainfall in the Mediterranean and the high aquifer exploitation have kept the water table at a low level for a long time. In order to improve the aquifer situation and to recover river quality, several activities were undertaken in the zone. These included reducing aquifer extractions, improving aquifer exploitation, improving the performance of the Castell-Platja d'Aro WWTP and promoting water reuse for irrigation. In 2003, after a rainy year, the aquifer finally recovered and the Ridaura River began to show continuous natural flows again for several months. Since then, these natural flows are happening every year, from autumn to spring.

Castell-Platja d'Aro is a municipality located in the Costa Brava area that has a WWTP and a reclaimed water facility (RWF). The WWTP performs a conventional activated sludge process which produces approximately 4.0 hm<sup>3</sup>/year of secondary effluent. Part of this effluent (2.4 hm<sup>3</sup>/year) is discharged into the Ridaura River, as the WWTP's submarine outfall capacity is limited (1.6 hm<sup>3</sup>/year), i.e. not all of the volume can be discharged into the Mediterranean Sea. The RWF began operation in 1998. It uses coagulation, sand filtration, and combined disinfection (UV light + chlorine) to treat the secondary effluent produced in the Castell-Platja d'Aro WWTP. Almost all of the reclaimed water, 0.75 hm<sup>3</sup>/year out of 1.0 hm<sup>3</sup>/year (the total volume produced), is used to supply water to several golf courses and agricultural areas including a corn field and an orchard. The water surplus (0.25 hm<sup>3</sup>/year) is discharged into the Ridaura River.

As a result, the microbiological quality of the Ridaura river downstream from the WWTP is affected by: i) the presence or absence of natural flows; ii) the discharges of secondary effluent and reclaimed water; and iii) the microbial dynamics of the river environment.

The aims of this study were: i) to describe WWTP performance, ii) to depict the current microbiological status of the Ridaura river, iii) to determine which changes in river microbiological quality are observed as a result of the WWTP discharges, and iv) to determine the potential improvements in river water quality that could be achieved by expanding the capacity of the water reclamation facility or increasing the capacity of the submarine outfall. This study therefore provides a sound basis for a decision-making process aimed at improving water quality, public health and environmental quality in the area.

## **MATERIALS AND METHODS**

### **Wastewater treatment plant description**

The WWTP uses a conventional activated sludge process for secondary treatment. A portion of the secondary effluent is subsequently treated in a nearby WRF, with a theoretical treatment capacity of 625 m<sup>3</sup>/hour. Tertiary treatment consists of four steps: coagulation, filtration (pulsed-bed sand filters), UV light disinfection (dose 24-36mJ/cm<sup>2</sup>), and post-chlorination (dose 3-5 mg Cl<sub>2</sub>/l to obtain a final concentration of 5ppm).

### **Sampling points and sampling strategies**

Secondary and tertiary effluent water samples were collected to evaluate the performance reclaimed water facility. Secondary effluent water samples (SEC) were taken after secondary decantation. Tertiary effluent water samples (TER) were taken after post-chlorination. Two sampling points were studied to evaluate river water quality: one point (P3) was located 6 Km before the WWTP flows into the river and the other (P5) 700 m after the WWTP flows.

In 2004-2005, two sampling strategies were adopted. The first involved random, regular sampling approximately every 2 months (n=11)(annual sampling) The second included an intensive sampling program undertaken in two consecutive weeks in the late spring of 2005, when the Ridaura river had no natural flows (n=10)(intensive sampling). The first sampling strategy considered sampling points P3, SEC, TER and P5. The aim was to i) evaluate the capacity of tertiary treatment to inactivate microorganisms; ii) document the current microbial quality of the Ridaura River; and iii) determine the influence of WWTP flows (mainly of secondary effluent) on river water quality. The second sampling strategy considered sampling points SEC, TER and P5. The aim was to assess the improvement in microbiological water quality downstream of the WWTP when almost all of the secondary effluent was derived to tertiary treatment and discharged as reclaimed water into the river. The collection and transport of samples to the laboratory were carried out according to ISO 5667-2 (Anonymous, 1991) and ISO 5667-3 (Anonymous, 2001).

### **Microorganisms detected**

All samples were analyzed for the presence of fecal coliforms (FC), spores of sulfite-reducing clostridia (SRC), somatic coliphages (SOMCPH), RNA F-specific phages (FRNAPH), phages that infect *Bacteroides fragilis* strain RYC2056 (BFRYCPH), phages that infect *Bacteroides thetaiotaomicron* strain GA-17 (BTGA17PH), cytopathogenic enteroviruses (EV) and total *Cryptosporidium* oocysts (CRYP).

### **Quantification of bacterial indicators**

Fecal coliform bacteria were quantified by membrane filtration using 47-mm cellulose acetate filters with a nominal pore size of 0.45 μm (Millipore EZ-Pack™ membrane filters, Cat. number: EZHAWG474). They were cultured on mFC agar (Difco, Sparks, MD) for 24 h at 44°C. SRC were

cultured on SPS agar medium for 24 h at 44 °C (Bufton, 1959). The detection limit was 10 cfu/100mL.

### **Quantification of bacteriophages**

SOMCPH, FRNAPH, BFRYCPH and BTGA17PH were enumerated by the double –agar layer method described by Adams (1959). The analysis followed ISO standards 10705-1 (Anonymous, 1995), 10705-2 (Anonymous, 2000) and 10705-4 (Anonymous, 2001). SOMCPH were enumerated using strain WG5 of *Escherichia coli*; FRNAPH were enumerated using strain WG 49 of *Salmonella thyphimurium*; and phages infecting *Bacteroides* were enumerated with the strains mentioned above. Between 100 and 250 mL of sample were concentrated when necessary, using the method described by Sobsey *et al.* (1982), as modified by Mendez *et al.* (2004). The detection limit was 1pfu/100mL or 0.4 pfu/100mL.

### **Quantification of cytopathogenic enteroviruses**

Buffalo green monkey kidney continuous cell line (BGM) (ECAAC 90092601) was used to enumerate cytopathogenic enteroviruses. BGM cells were grown in Eagle's minimum essential medium (MEM) (ICN Biomedicals, Inc. Aurora, Ohio) containing 5% fetal bovine serum (FBS), 2 mM L-glutamine, 26.8 mM NaHCO<sub>3</sub>, 100 U/mL of penicillin and 100 µg/mL of streptomycin. Secondary effluent water samples (20 mL) were analyzed by the double layer plaque assay (Mocé-Llivina *et al.*, 2004). Tertiary effluent water samples and river water samples (50-100L) were filtered through MK cartridges (AMF Corp. Cuno Division, Meriden, CN, USA). Viruses were eluted with 0.25M glycine buffer solution pH 10.5 for 25 minutes. A secondary concentration step involved organic flocculation, as described by Katzenelson *et al.* (1976). The concentrate (50 mL) was decontaminated as described in Mocé-Llivina *et al.* (2003) and analyzed using the double layer plaque assay method (Mocé-Llivina *et al.*, 2004). The detection limit was 50 pfu/L in secondary effluents and 0.01 or 0.025 pfu/L in tertiary effluents.

### ***Cryptosporidium* detection and enumeration**

Secondary effluents (1L) were processed as described by Shepherd *et al.* (1995), with modifications as detailed in Montemayor *et al.* (2005). Tertiary effluent samples (20-100L) were concentrated using Envirochek™ sampling capsules (Pall Gelman Laboratory, Ann Arbor, MI), according to Method 1623 (U.S. EPA, 1999). Oocysts were visualized by laser scanning cytometry, as described in Montemayor *et al.* (2005). The detection limit oscillated according to the water volume concentrated.

### **Physical-chemical parameters**

Physicochemical parameters of secondary and tertiary effluents were analyzed during both sampling strategies. Turbidity was determined in a DINKO D-112 turbidimeter; the percentage of transmittance at 254 nm was measured in a UNICAM Helios Gamma spectrophotometer; pH in a CRISON PH25 pHmeter; and conductivity in a CRISON 524 conductivity meter. Total suspended solids (TSS), ammonia and phosphorus concentrations were measured as described in Standard Methods 2450-D, 4500-NH<sub>3</sub>E and 4500-P C respectively.

### **Statistical analysis**

The Statistical Package for Social Sciences (SPSS for Windows 14.0, Chicago, IL, USA) and Statgraphics Plus for Windows 5.1 (Statistical Graphics Corp.) were used for statistical analysis. Samples with negative microbial counts were included in the statistical analysis; as the microbial concentrations in such samples gave the value of the detection limit.

## RESULTS AND DISCUSSION

### WWTP performance

Secondary and tertiary effluents taken using both sampling strategies met the required physicochemical standards ( $BOD_5 < 25\text{mg/l}$ ;  $TSS < 35\text{mg/l}$  for secondary effluents and  $TSS < 20\text{mg/L}$ ; turbidity  $< 5\text{NTU}$  for tertiary effluents) for being discharged into the environment. Values of the physicochemical parameters are shown in Table 1.

**Table 1.** Average values and standard deviations for the physicochemical parameters analyzed during annual sampling and intensive sampling.

Sampling point	TSS mg/L		Turbidity NTU		T254 %		pH	CE dS/m	Amonium	Phosphorus
	SEC	TER	SEC	TER	SEC	TER			mg NH <sub>3</sub> - N/L	mg P/L
N Annual sampling	19	18	19	18	19	18	19	19	19	19
Average Annual Sampling	7.5	3.7	3.6	2.2	57.6	54.6	7.7	1.2	20.0	4.1
DESVEST Annual sampling	6.4	3.0	4.6	3.0	9.8	13.1	0.3	0.2	12.2	2.5
N Intensive sampling	6	6	6	6	6	6	6	6	6	6
Average Intensive Sampling	9.7	4.4	3.4	2.4	49.2	40.8	7.6	1.2	32.5	6.0
DESVEST Intensive Sampling	4.4	2.2	1.7	0.9	7.6	12.3	0.1	0.1	4.4	2.5

SEC: secondary effluent water samples; TER: tertiary effluent water samples; TSS: total suspended solids; CE: electric conductivity; T245%: percentage of transmittance measured at  $\lambda=245\text{nm}$

There were no significant differences (ANOVA test  $p\text{-value} > 0.05$ ) in the microbial concentrations or microbial inactivation achieved in tertiary treatment between microbial data obtained during annual sampling and that obtained during intensive sampling. Therefore, both data sets were treated as one. Figure 1 shows the geometric mean concentrations, expressed as  $\log_{10}$  units, of indicator microorganisms in secondary and tertiary effluents. The number of samples analysed, the percentage of positive samples, the minimum and maximum values and the average concentrations of all parameters are shown in Tables 2 and 3.

**Table 2.** Percentage of positive samples, minimum, maximum and mean values of all the microorganisms studied in the secondary effluent of the Castell-Platja d'Aro WWTP.

Microorganisms	Number of samples	Positives %	Minimum	Maximum	Geometric mean Log 10 units
FC, cfu/100 ml	21	100	10000	2000000	5.4
SRC, cfu/100 ml	21	100	1200	42000	3.8
SOMCPH, pfu/100 ml	21	100	2600	220000	4.7
FRNAPH, pfu/100 ml	21	100	1600	73200	4.0
BFRYCPH, pfu/100 ml	21	90	<100	550	1.8
BTGA17PH, pfu/100 ml	20	100	30	3150	2.5
CRYP, oocysts/L	11	91	<1	57	0.8
EV, pfu/L	11	36	<50	250	1.3

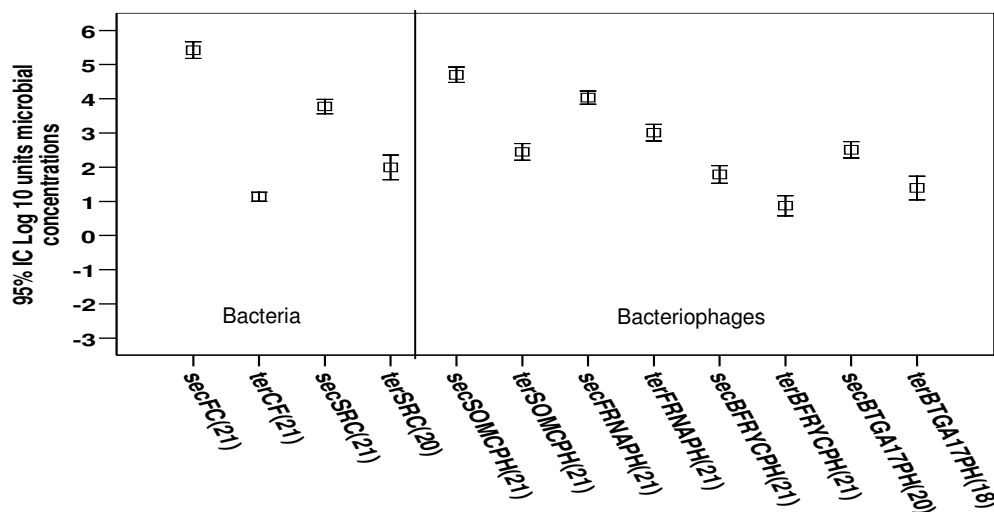
Studies of conventional activated sludge treatment plants located in different geographical areas showed similar microbial concentrations to those presented in this work (Lucena *et al.*, 2004; Lodder *et al.*, 2005). Tertiary treatment affected more bacteria than bacteriophages. This is relevant, as quality disinfection controls are based on fecal bacteria quantification (WHO Guidelines 1989, USEPA 2004). In this study, fecal bacteria were the least resistant to treatment. The geometric mean

of microorganisms' inactivation was  $\geq 4.32 \log_{10}$  units for FC;  $\geq 1.98 \log_{10}$  units for SRC;  $\geq 1.28 \log_{10}$  units for EV;  $\geq 1.25 \log_{10}$  units BFRYCPH;  $\geq 0.63 \log_{10}$  units for CRYP; 2.30  $\log_{10}$  units for SOMCPH; 1.29  $\log_{10}$  units for BFGA17PH; and 1.18  $\log_{10}$  units for FRNAPH. Pathogen reductions were similar to those observed for bacteriophages. FRNAPH were the most resistant of the phages. This may be due to their higher resistance to UV light disinfection (Hijnen *et al.*, 2006).

**Table 3.** Percentage of positive samples, minimum, maximum and average values of all the microorganisms studied in the tertiary effluent of the Castell-Platja d'Aro WWTP.

Microorganisms	Number of samples	Positives %	Minimum	Maximum	Geometric mean Log 10 units
FC, cfu/100 ml	21	33	<10	150	1.1
SRC, cfu/100 ml	20	75	10	900	1.9
SOMCPH, pfu/100 ml	21	100	40	1700	2.5
FRNAPH, pfu/100 ml	21	100	100	6880	3.0
BFRYCPH, pfu/100 ml	21	57	<1	88	0.9
BTGA17PH, pfu/100 ml	18	83	1	384	1.4
CRYP, oocysts/L	10	90	0.02	58.2	0.4
EV, pfu/L	11	27	0.01	0.02	-1.9

**Figure 1.** Geometric mean concentrations and 95% confidence levels of bacterial indicators and bacteriophages in the secondary and tertiary effluent of the Castell-Platja d'Aro WWTP. Between brackets is the number of samples analyzed in each case. FC and SRC are expressed in cfu/100mL. SOMCPH, FRNAPH, BFRYCPH and BFGA17PH are expressed in pfu/100 ml.



### Changes in microbiological quality of the stream

The number of samples analysed, the percentage of positive samples, the minimum and maximum values and the mean concentrations of all the parameters studied are shown in Table 4. Fecal coliform concentrations in P3 were compared with the FC concentrations observed in the origin of other rivers located in our geographical areas and available at the Catalan Water Agency website. Both levels were similar, which indicates that the microbiological water quality of the Ridaura River is comparable to the headwaters of other Catalan rivers.

**Table 4.** Percentage of positive samples, minimum, maximum and mean values of all the microorganisms studied in the Ridaura River sampling points P3 and P5, both during the annual sampling (A) and the intensive sampling (I) periods.

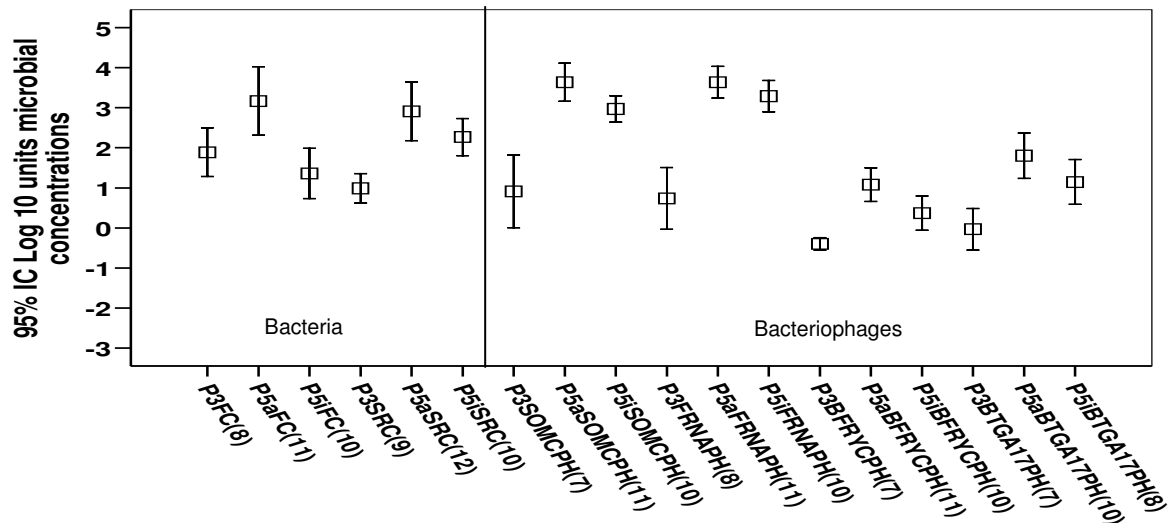
Microorganisms	Sampling point	Number of samples	Positives %	Minimum	Maximum	Geometric mean Log 10 units
FC, cfu/100 ml	P3	8	100	8	950	1.89
	P5A	11	82	<10	150000	3.17
	P5I	10	30	<10	5300	1.36
SRC, cfu/100 ml	P3	9	13	<10	80	0.99
	P5A	12	100	10	90000	2.91
	P5I	10	90	<10	1700	2.27
SOMCPH, pfu/100 ml	P3	8	38	<1; <0.4	400	0.91
	P5A	11	100	420	55000	3.64
	P5I	10	100	200	5600	2.97
FRNAPH, pfu/100 ml	P3	8	25	<1; <0.4	140	0.74
	P5A	11	100	160	24800	3.64
	P5I	10	100	300	24800	3.29
BFRYCPH, pfu/100 ml	P3	8	0	<0.4	<0.4	-0.4
	P5A	11	91	<1	100	1.08
	P5I	10	40	<1	32	0.37
BTGA17PH, pfu/100 ml	P3	7	29	0.4	12	-0.03
	P5A	10	100	2	1050	1.8
	P5I	8	78	<1	109	1.15
CRYP, oocysts/L	P3	5	20	<0.025	0.09	-1.22
	P5A	7	14	<0.08	54	0.18
	P5I	4	100	0.56	84	1.01
EV, pfu/L	P3	5	40	<0.025	5	-0.93
	P5A	7	57	<0.01	3	-1.01
	P5I	4	25	<0.01	0.01	-1.98

Microbial values obtained in P3, with the exception of those obtained for ENT, were different from those obtained in P5A (ANOVA test  $p$ -value>0.05). This indicates that WWTP flows (mostly secondary effluent) deteriorate the microbiological stream water quality (Figure 2). Microbial concentrations were different when the river flowed naturally and when it did not. However, these differences were not significant (ANOVA test  $p$ -value >0.05). As the Ridaura River is a temporary stream, the diluting factor should not have to be considered to evaluate the impact of WWTP flows.

Intensive sampling showed that reclaimed water flows improve microbiological river water quality. In this study, there were no statistically significant differences between microbial concentrations in P5 during annual sampling and intensive sampling (ANOVA test  $p$ -value >0.05). This could be explained by the irregularity in raw water entrance to WWTP, producing two flow increases per day. During these increases, secondary effluent volume is higher than the volume that submarine outfall can discharge into the sea and the volume that the reclaimed water facility can treat.

Therefore, there is a discharge of an undetermined volume of secondary effluent water into the river which affects the river water quality.

**Figure 2.** Geometric mean concentrations and 95% confidence levels of bacterial indicators and bacteriophages in sampling points P3 and P5 of the Ridaura River (before and after WWTP discharge, respectively). Between brackets is the number of samples analyzed. FC and SRC are expressed as cfu/100mL. SOMCPH, FRNAPH, BFRYCPH and BFGA17PH are expressed in pfu/100 ml. P5a represents the value obtained during annual sampling; P5i represents the value obtained during intensive sampling.



## CONCLUSIONS

The microbiological quality of the Ridaura river is affected by the discharge of the secondary effluent of the Castell-Platja d'Aro WWTP. The sampling point above the discharge point shows a good microbiological quality, similar to that of the headwater of other Catalan rivers, whereas the sampling point below the discharge point remains of similar quality even when tertiary effluent is discharged, except for the fecal coliforms. The reduction of the secondary effluent discharges by increasing the capacity of the submarine outfall or that of the reclaimed water facility would help to reduce the effect of WWTP flows into the stream and to protect public health. Moreover, in order to assure public health protection, more resistant microorganisms than FC, such as coliphages, should be considered in quality controls.

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