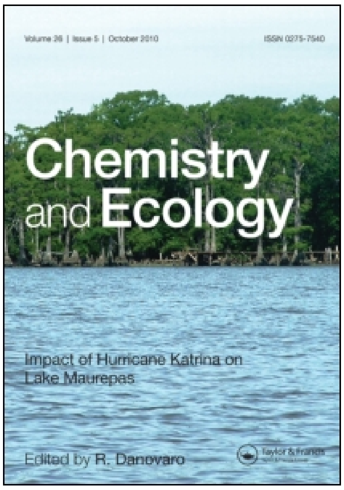

Chemistry and Ecology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455114

Intergrated approach for microbiological impact assessment of public wastewater treatment plants

Marco Ostoich[a]; Emilia Aimo[b]; Rita Frate[b]; Marina Vazzoler[c]; Silvia Stradella[b]; Paolo Osti[d]
[a] Inland Waters Observatory, Provincial Department of Padua, ARPAV (Veneto Regional Prevention and Protection Agency), Padua, (PD), Italy [b] Venice Provincial Department, ARPAV, Mestre, (VE), Italy [c] Northern Adriatic Sea Observatory, ARPAV, Padua, (PD), Italy [d] Environmental Policies Dep., Province of Venice, Mestre, (VE), Italy

Online publication date: 21 September 2010

To cite this Article Ostoich, Marco , Aimo, Emilia , Frate, Rita , Vazzoler, Marina , Stradella, Silvia and Osti, Paolo(2007) 'Intergrated approach for microbiological impact assessment of public wastewater treatment plants', Chemistry and Ecology, 23: 1, 43 — 62

To link to this Article: DOI: 10.1080/02757540601083963
URL: http://dx.doi.org/10.1080/02757540601083963

Chemistry and Ecology
Vol. 23, No. 1, February 2007, 43–62

Intergrated approach for microbiological impact assessment of public wastewater treatment plants

MARCO OSTOICH*†, EMILIA AIMO‡, RITA FRATE‡, MARINA VAZZOLER§, SILVIA STRADELLA‡ and PAOLO OSTI¶

†ARPAV (Veneto Regional Prevention and Protection Agency), Inland Waters Observatory, Provincial Department of Padua, Piazzale Stazione 1, 35131 Padua (PD), Italy
‡ARPAV, Venice Provincial Department, via Lissa 6, 30171 Mestre (VE), Italy
§ARPAV, Northern Adriatic Sea Observatory, Piazzale Stazione 1, 35131 Padua (PD), Italy
¶Province of Venice, Environmental Policies Dep., via Forte Marghera 191, 30173 Mestre (VE), Italy

(Received 26 September 2005; in final form 27 September 2006)

Wastewaters are a source of pathogenic micro-organisms in the environment. The microbial load and residues found in the final effluents of wastewater-treatment plants (WWTPs) depend on the WWTPs' abatement capacity and the final disinfection treatment systems applied to wastewaters before discharge into water. A historical database with data on surface and marine-coastal water quality and on the characterization of WWTP effluents was made using data from 1997 to 2004 to assess the microbiologic impact along the coast of the Venice province (Italy, northern Adriatic sea). The monitoring of river and sea discharges along the coast is integrated with the application of the *Synthetic Pluriennal Faecalization Index* (ISPF). The experimental study was conducted in the period from November 2002 to April 2004 by the Veneto Regional Environmental Prevention and Protection Agency. The results of this investigation on faecal contamination together with previous data are presented with a preliminary performance characterization of the WWTPs' disinfection technologies (sodium hypochlorite, peracetic acid, UV rays, and ozone).

Keywords: Total and faecal coliforms; Faecal streptococci; *Escherichia coli*; Enterovirus; Cytopathogenic virus; Integrated analysis

1. Introduction

The protection and safeguarding of water quality are among the most important priorities because of implications for the environment and human health in areas where there could be direct or indirect contact (ingestion, aerosol/liquid inhalation, epidermal contact, etc.) with pathogens. This is enhanced in coastal areas [1], characterized by high urbanization, high densities of recreational facilities, and sources of faecal pollution from both human and animal wastes. Generally, the same localities are uniquely productive, valuable, and fragile environments. Worldwide now, there is increasing pressure on coastal areas due to urbanization, and chemical and microbiological pollution [2].

*Corresponding author. Email: mostoich@arpa.veneto.it

Chemistry and Ecology
ISSN 0275-7540 print/ISSN 1029-0370 online © 2007 Taylor & Francis
http://www.tandf.co.uk/journals
DOI: 10.1080/02757540601083963

In this work, the results of a large survey on the microbiological impact in the coastal area of the Venice province are presented and discussed according to previous investigations [3]. A database has been made on the microbial components collected in freshwater, wastewater-treatment plants (WWTPs) and Marine Coastal waters. The objectives of this study are:

- to define the level of microbiological contamination in the coastal area of the Venice province;
- to gather information on the effects of faecal wastewaters on the final rivers estuaries and on the coast of the Venice province;
- to make available a complete, systematic, and up-to-date database on water and discharge quality;
- to make a preliminary comparison between different wastewater disinfection systems and their effectiveness in microbiological abatement;
- to develop an *integrated quality assessment* of rivers, marine and coastal waters, and wastewaters for coastal management.

The treatment plants were selected from those with historical data and reported in the list of the local authority of the Venice province. Of the 10 chosen plants, nine were public WWTPs with CH_3COOOH, UV radiation, and NaOCl disinfection systems. Despite its indirect effects on marine waters, one plant, an industrial private plant, was selected because this was the only example of an ozone-disinfection system.

This paper attempts to provide a preliminary comparison between the different disinfection technologies: the evaluation is limited as no disinfectant dosage and quantification of contact time have been supplied. The data are compared under the hypothesis that the operative conditions are the same as those in the WWTPs considered.

The study is a comprehensive analysis similar to other studies reported in the scientific literature on the microbiological impact of WWTPs [2, 4–8] but applied in a specific Italian (northern Adriatic sea) context, according to the integrated approach established by Directive 2006/7/EC [9]. Further studies are necessary to supply more data for the comparison of disinfection technologies, with data on disinfection dosage and on contact time; it is also necessary to investigate the management conditions of the WWTPs considered.

1.1 *Microbiological impact on water in the coastal areas: pathogens and faecal indicators*

Most water-borne pathogens, originating from human and animal faeces, include a wide variety of bacteria and viruses [10]. Wastewaters are a source of pathogens and not pathogens [11, 12] and they can be point or non-point sources [13]. The presence of specific pathogens reflects the health of the human or animal population which generated the wastewaters [12].

Faecal coliform bacteria are widely used as indicator organisms to signal the possible presence of faeces and pathogens [2] whereas *faecal coliforms* do not reliably predict the occurrence and survival of enteric viruses [14, 15]. Zann and Sutton [6] suggest as an indicator of faecal pollution: *faecal coliforms* (FC) and/or *faecal streptococci* (FS). Bacterial groups of FC and FS correlate reasonably well with some of the bacterial pathogens such as the *Salmonellae* [16, 17].

Total coliforms (TC), FC, FS, *Escherichia coli* (EC), and Enterococci are used as bacterial indicators for water-quality monitoring and health assessment as they are much easier and less costly to detect and enumerate than the pathogens themselves [18]. Pathogen detection is difficult because bacteria and viruses can be associated with particulate material, and there are problems of die-off prediction [6].

The FC as indictor of enteric bacterial and viral pathogens is not proven, but is still largely used [19]. The different microbial species and their relative concentrations are restricted to the local epidemiological situations and to the amounts abatement of pollutants achieved on wastewaters.

With reference to disinfection systems, Zann and Sutton [6] point out that it is important to note that although a 99.9% reduction in pathogens may at first appear satisfactory, this is often not enough. In fact, the discharge of non-disinfected raw or primary/secondary sewage effluents into bathing waters is expected to represent a local health risk without any further dilution/die-off of at least 1000-fold, as can occur through deepwater sea outfall. Grohmann *et al.* [20] observed a high incidence of positive cases for enteric viruses in Sydney on beach waters prior to commissioning three deepwater ocean outfalls. EC is recommended as an indicator of faecal contamination in freshwaters [12, 21]. Sinton *et al.* [22] suggest the FS as a faecal pollution indicator. Other studies suggest FC and FS as faecal indicators for water quality, and *Campylobacter* and *Salmonella* for the presence of pathogens [7]. EC and Enterococci are indicated [9] for the assessment of faecal contamination from sewage-treatment facilities.

As can be seen from previous references from scientific literature on the topic, there is no single indication of which parameter or parameters should be used as the best indicators of faecal pollution.

1.2 *European approach to the management of bathing water quality*

In Italy, the bathing guidelines presently in force [23], which enforced the previous Directive on bathing waters [24], prescribe maximum concentrations of FC, TC, FS, and EC for human recreational use for the microbiological quality of coastal waters. The new European Directive 2006/7/EC on management of bathing-water quality has drastically reduced the number of parameters from the previous 19 to two key microbiological parameters. This directive aims to establish more reliable microbiological indicators.

The policy on bathing waters must satisfy the general objective of ‘good ecological status’ expressed in the 2000/60/EC Water Framework Directive [25] to be achieved with river-basin management plans and programmes of measures, and must follow a new approach based on an integrated management of water quality.

The two faecal indicator parameters retained in Directive 2006/7/EC are Intestinal Enterococci (IE) and EC, providing the best match between faecal pollution and health impacts in recreational waters according to available scientific evidence provided by epidemiological studies.

1.3 *Importance of tourism and bathing in the local economy*

The importance of the control and monitoring of coastal marine waters [26] is particularly evident in the Venice province, where many important tourist sites are located. Furthermore, the economic and urban development of the province is responsible for significant discharges both into rivers and into marine waters, with the need for efficient WWTPs. Therefore, the sanitary and environmental ‘quality’ of the coastal belt of the Province is very important from both an environmental and economical point of view (tourism).

The population on the coastal area of the Venice Province numbered some 392 617 in 2001 (last official census [27]) and tourists in the coastal territory of the seven municipalities (San Michele al Tagliamento, Caorle, Eraclea, Jesolo, Cavallino-Treporti, Venezia, Chioggia) in 2001 and 2002 numbered 27 780 868 and 26 756 310 per year, respectively (source: Veneto Region, 2001 and 2002).

The number of marine-coastal waters not complying with Italian bathing law [23], in terms of the microbiological parameters for 2002 and 2004 (source: Veneto Region-ARPAV, 2004, internal report) can be summarized as follows, according to municipality:

- year 2002: bathing banned at 11 sites (four in Caorle, one in Jesolo, and six in Chioggia municipalities, forbidden from a minimum of 3 d to a maximum of 66 d during the bathing season);
- year 2004: bathing banned at six sites (one in Caorle and five in Chioggia municipalities from a minimum of 2 d to a maximum of 155 d during the bathing season);
- in the Chioggia municipality, in 2004 (from year 2003 data), two monitoring sites banned bathing for the whole year; in 2005 (from year 2004 data), five monitoring sites banned bathing.

2. Materials and methods

2.1 *Integrated assessment for coastal management*

The application of the approach expressed in the new European legal framework (directive 2000/60/EC and directive 2006/7/EC) requires that a distinct analysis be undertaken for each single matrix (rivers, marine waters, WWTP effluents, etc.). The new approach favours *integrated quality assessment* of the separated components of the territorial hydro-systems, analysed for their reciprocal relationships in accordance with the Driving forces-Pressures-State-Impact-Responses (DPSIR) model. The DPSIR model, proposed by the European Environmental Agency [28], was derived from the simpler Pressure-State-Responses model [29], for which there are many applicable examples for waters in the literature [30–32].

To protect sea resources from enteric-bacteria pollution, coming from river flow and WWTP discharges, environmental management and safeguarding practices must be defined and implemented.

The study proposes an approach based on the recovery of historical databases of data from WWTP controls, (on rivers, bathing, coastal, and marine waters) and on the assessment of the Synthetic Pluriennal Faecalization Index, for the integrated quality assessment of microbiological impact, using the DPSIR model. From investigations on the presence of microbial and viral organisms in inflow and outflow wastewaters, it has been possible to verify the efficiency of different disinfection technologies applied to selected WWTPs.

The integrated quality assessment is presented here as a preliminary to the more comprehensive Integrated Coastal Management (ICM) [32–34]. In this paper, the modelling evaluation of pollution diffusion and the institutional governance system are not developed; the environmental evaluation is referred only to the microbiological impact analysis, as this is particularly critical for bathing-water quality and consequently for the local economy. Microbiological impact is a fundamental component of ICM as coastal areas are heavily used for recreational and economic activities. The study proposes faecal indicators based on technical legislation and an integrated assessment in support of ICM.

2.2 *Sampling stations*

The selected area for the study is localized all along the coast of the Venice province, and the following control points were considered:

- existing monitoring sites for surface waters (rivers and marine-coastal waters) along the coast (ca 500 m from the beach);
- WWTPs, known to influence the marine-coastal waters, and a private treatment plant.

Table 1 and figure 1 report public WWTPs and one private industrial discharge; monitoring stations for surface internal waters; monitoring points for bathing waters; and for marine waters, considered in the Venice province. In table 1, sampling stations in which integrative samples were collected in the present study are reported in bold, while the remaining stations are referred to as historical data. The coast was divided into different areas, each connected to

Table 1. Subdivision of the Venice Province coast in areas and correspondence with monitoring stations for surface, bathing, and coastal waters (these last ones 500 m from the beach were active until 2001 and then modified) with WWTPs.

Area	Reference river	Freshwater monitoring stations	WWTP (provincial code and name)	Bathing monitoring stations	Marine-coastal monitoring stations*
I	Tagliamento river	78, **432**	1	517, 002, 003, 004, 005, 518, 007	101, 108
II	Lemene river	**71**, 75, 76, **433**	3	008, 009, 519, 010, 011, 012	110
III	Livenza river	**72**, 151	2	013, 014, 520, 521, 015, 498, 016, 017	115
IV	Piave river	**65**, 152	4	018, 019, 020, 499, 021, 022, 023, 024, 025, 026	124
V	Sile river, Sile-old Piave river	**237**, **238**	5, 6	027, 028, 029, 030, 032, 033, 034, 035, 036, 075, 037, 500	132
VI	Venice Lagoon San Nicolò mouth (no rivers)		7	038, 039, 040, 041, 526, 042, 043, 044, 045, 046, 047, 048, 049	140, 147
VII	Venice Lagoon Malamocco mouth (no rivers)		8	501, 502, 050, 051, 052, 053, 054, 055	153
VIII	VIIIa: Brenta mouth	**436**, **437**, 212	9	503, 056, 057, 058, 059, 060, 061, 522, 523, 063, 064, 065, 066, 524	156, 159, 162, 164
	VIIIb: Adige mouth	**217**, **222**			

Note: Source: Veneto Region-ARPAV. *In the figure 1 the last two digits are included to indicate the transects of the monitoring stations along the coastline.
For the localization of monitoring points and WWTPs, see figure 1.

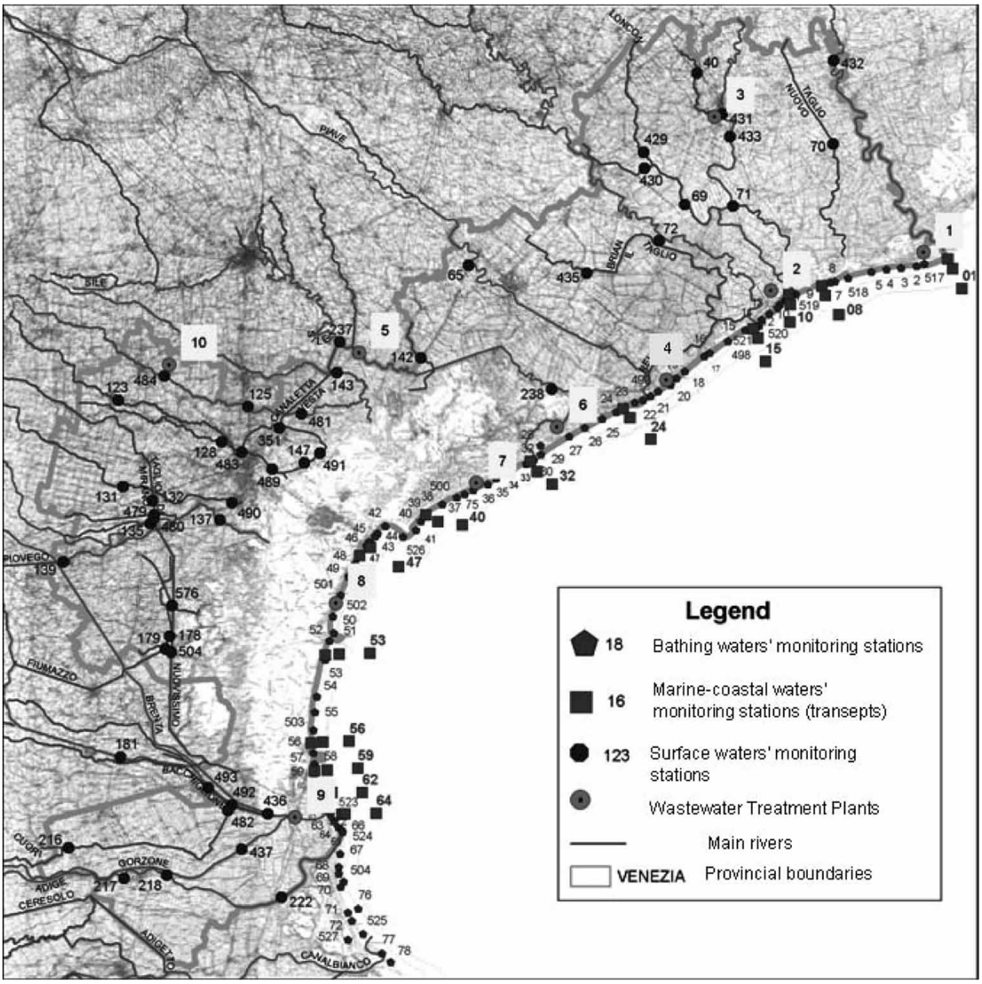

Figure 1. Localization of monitoring stations of surface internal waters, bathing water monitoring stations, marine water monitoring stations (from 500 m from the coast—stations operating until 2001), and WWTPs. For the corresponding numbers of the monitoring stations, see table 1. Sources: Veneto Region-ARPAV and Province of Venice.

a river body (in two cases, Lido and Cavallino, the coast does not present a river mouth) and which could be considered homogeneous for the environmental characterization according to physical and geographical aspects.

A set of historical data from 1997 (1991 for marine waters) to 2004 for surface-water quality, marine-coastal water monitoring, and characterization of WWTP discharges was extracted from the Regional Environmental Informative System (SIRAV) managed by ARPAV and integrated with other data. New chemical, microbiological, and virological analysis tests were conducted at the rivers monitoring stations and on the selected WWTPs (inflow and outflow wastewaters) in the period 2003–2004.

2.3 *Sampling and analyses: parameters and reference methods for microbiology*

A complete and up-to-date database was realized with data from monitoring and controls (1 January 1997 to 30 September 2004) for assessment. The database was used for the assessment of the degree of pollution; the WWTP integrative data (in and out) from the experimental campaign (2003–2004) were used to assess the efficiency of the different disinfection technologies. The samples of microbiological parameters were analysed according to the following procedures:

- influents of WWTPs: instantaneous sampling at the arrival of the sewage pipe;
- effluents of WWTPs: instantaneous sampling for virological parameters, mean-composite sampling on 24 h for other parameters;
- surface waters: instantaneous sampling for all parameters.

For WWTPs, the wastewater flows and homogeneity characteristics allowed us to correlate influent and effluent data. For the objectives of the study, each surface water or wastewater sample was analysed for the following parameters:

- quali-quantitative determination of TC, FC, EC, FS and *Salmonella* (absence/presence with characterization of the serotype);
- research on 'enteric virus' with cytopathogenic effects and identification of enterovirus on positive samples for cellular farming with molecular-biological techniques.

Table 2 summarises the various biological parameters investigated and reference methods applied.

For research on TC, FC, FS, and EC samples, 500 ml of surface waters or wastewaters was collected. The analytical methods followed were:

- *Total coliforms*: determined according to the standard procedure [35] and expressed as colony-forming units (cfu) per 100 ml.

Table 2. Microbiological parameters and reference methods.

Parameter	Meaning	Reference method
Total coliforms	Heterogeneous groups of the Enterobacteriacae group of faecal and/or environmental derivation. Useful as indicators of the efficiency of water depuration systems and of integrity of the water pipes.	Report: ISTISAN 97/8 [35]
Faecal coliforms	Indicator of faecal contamination of human and animal origin. Indicator of the water treatment systems for drinking-water.	Report: ISTISAN 97/8 [35]
Escherichia coli	Indicator of faecal contamination of human and animal origin.	Analytical methods for water Vol. 3, 29/2003, APAT IRSA-CNR, method f [36]
Faecal streptococci	Indicator of faecal contamination of human and animal origin. Indicator of the water treatment systems for drinking-water.	Report ISTISAN 97/8 [35]
Salmonella	Pathogen of human and animal faecal origin, adapted to a specific host or ubiquitous.	Report: ISTISAN 00/14 Pt. 2 [37]
Enteric virus	Virus present in the excrements. Endocellular ultra-microscopic parasites, without the necessary organization for replication; for this reason virus present in the aquatic environment derive only from faecal contamination (*Enterovirus*, HAV, HEV, *Norwalk* virus, *Rotavirus*, *Reovirus*, *Adenovirus*).	Report: ISTISAN 00/14 Pt. 2 [37]
Enterovirus	Group of enteric virus constitued by: *Poliovirus* 1–3, *Coxackievirus* A e B, *Echovirus* 1–34 and other enteroviruses.	Report: ISTISAN 00/14 Pt. 2 [37]

- *Faecal coliforms*: determined according to the standard procedure [35] and expressed as cfu per 100 ml.
- *Faecal streptococci*: determined according to the standard procedure [35] and expressed as cfu per 100 ml.
- *Escherichia coli*: determined according to the standard procedure [36] and expressed as cfu per 100 ml.
- *Salmonellae*: determined according to the standard procedure [37]. The method allows only qualitative results and typization of *Salmonella*; the analytical procedure is carried out in spring water, groundwater, surface water, and water for human consumption. For *Salmonella*: 1 l of surface water or wastewater sample is used. The analytical procedure allows the presence/absence of the pathogen to be evaluated through successive phases: pre-enrichment, enrichment, isolation, biochemical, and serological confirmation.
- *Enteric viruses*: determined according to the standard procedure [37]. Most of the over 120 classified enteric viruses are very difficult to isolate from the aquatic environment, and many are simply non-cultivable [38]. Viruses which specifically infect bacteria (bacteriophages) are useful surrogates for human enteric viruses in survival studies [39]. Owing to the considerable variability in enumerating enteric viruses, results are generally discussed on a presence/absence basis [6].
- *Enteroviruses*: determined according to the standard procedure [37]. The method for the investigation (isolation and identification) of enteroviruses from environmental samples includes different systems: incubation on cellular cultivation, immunological systems (direct and indirect immuno-fluorescence, immuno-enzymatic tests, and radio-immunological tests), and molecular systems.

10 L of surface waters and 1 L of row wastewater were collected for the analyses of viruses. The detection method is of four phases: concentration, decontamination, isolation, and identification:

(1) *Concentration*. Ultra-filtration with tangential flow: separation process of particles according to the molecular weight with polisulfone membranes of molecular dimension 10 000 nominal molecular weight limit subject to conditioning with 3% flesh extract at pH 7 and final recovery of eventual viral particles adsorbed with 3% flesh extract at pH 9.
(2) *Decontamination*. Filtration with membrane of 0.22 μm, subsequent addition of an antibiotic–antimycotic pool in a 1:50 proportion, contact for 3 h at 37 °C, and conservation of the sample at a temperature below −20 °C until the moment of the inoculation.
(3) *Isolation*. Sowing of the sample on flowing cellular monolayers fit for their replication: Buffalo Green Monkey and Human Hepatocarcinoma-2. The appearance of the cytopathic effect on the cellular monolayer indicates the vitality and the infective role of the virus.
(4) *Identification of enterovirus*. The enterovirus-identification systems are the direct immuno-fluorescence and the biomolecular test reverse-transcriptase polymerase chain reaction (PCR). The PCR product is visualized with electrophoresis separation on an agarose gel with 2% ethidium bromide from part of the reaction mixture. This is run side by side with a molecular-weight marker to localize the band corresponding to the amplified region.

2.4 *Pathogens*

2.4.1 *Salmonella* spp. The genus *Salmonella* includes bastoncellar, rod-shaped micro-organisms belonging to the Enterobacteriaceae family that are Gram-negative, generally mobile with whips, and optionally anaerobic. Salmonella spp are identified by their serological characteristics that differentiate between about 2000 types and serotypes. Salmonellae were investigated in samples of surface waters and in the WWTP discharges according to the procedure of the Italian Chief Health Institute [37].

Bacteria belonging to the genus *Salmonella* live in the gastrointestinal habitat in humans and animals (both warm- and cold-blooded animals). On the basis of the adaptation capabilities, the *Salmonellae* are divided into two groups: (1) those that fit to a well-defined host; (2) those without a specific host, belonging to ubiquitous serotypes. Some strictly human serotypes are responsible for typhoid infections, while the ubiquitous serotypes cause clinical forms characterized by a lower pathogenic capability and localized at the intestinal level.

Among the *Salmonellae* adapted to a particular host, there are the following examples: *S. typhi*, which infects exclusively man and is responsible for typhoid fever; *S. gallinarum*, *S. abortus ovis*, and *S. abortus equi*, which are able to adapt themselves to the same animal species. The ubiquitous serotypes are characterized by a smaller pathogenic potential than *S. typhi* and are responsible for gastroenteric diseases. In water, the survival of *Salmonella* depends on complex ecological interactions between micro-organisms and the environment. Its presence in water environments certainly represents the existence of a primary faecal contamination (direct inflow of sewer discharges) or secondary faecal contamination (washing of contaminated soils).

The concentrations of pathogens, in waste, marine and fresh-waters are strickly connected with the human pathologies along the sampling areas and display seasonal variations. The hypothesis of a casual distribution of the micro-organisms is generally accepted, but more often they are associated with dissolved and particulate solids and this modifies their distribution in waters. There are many methods of transmission to humans, such as through the food chain, water environments, and inter-human infections. *Salmonella* can be detected in humans with enteric fevers, gastroenteritis, septicaemia, and typhus. The infecting dose is variable in relation to the serotype (10^7–10^9) [37]; *Salmonella* is widely diffused in environments where it can survive.

2.4.2 Virus. The Italian regulatory system, with the acknowledgement of the EC directives, introduced the research of the parameter ‘enterovirus’ among the hygienic–sanitary aspects on the microbiological component. Official references on viruses concern bathing waters [40] and on water for human consumption [41]. Investigations of enteroviruses in the environment offer the opportunity to assess infective risk associated with direct or indirect contact with different environmentally contaminated matrices.

Viruses are ultra-microscopic parasites without necessary organization for replication. It is believed that the common enteroviruses (*Poliovirus*, *Echovirus*, *Coxsakie virus*), even if they are found in drinking-waters, are not responsible for pathologies evident in users, either for the low doses that can be associated with drinking-water or for the high degree of immune coverage of the population. Therefore, it was concluded that these viruses are important as water-quality indicators. There is, moreover, the certainty that other viruses, defined more generically as ‘enteric’, have a fundamental role in the diffusion of pathologies, ranging from infective hepatitis to generic gastroenteritis. To investigate viruses in the environment, it can be necessary therefore to better define environmental quality in terms of viruses acting as ‘biological indicators’ [42].

The ‘enterovirus’ is a genus that belongs to the Picornaviridae family of the group of enteric viruses. These viruses are excreted with excrements and urines. Like all the virus, they are obliged endo-cellular parasites because they are devoid of the necessary organization for replication; it is important to point out that viruses are pathogens able to infect humans at very low concentrations. Enteric viruses enter the environment through wastewaters discharged into receiving water bodies or through sewage and sludge spread on the soil; therefore, waters contaminated by viruses can become sources of infections.

Virus survival in waters depends on many factors: natural capacity of self-depuration, physical factors (sunlight exposure, temperature, and presence of particulate matter), chemical factors, and biological factors. The viruses are also resistant to disinfection agents like NaOCl, used in the disinfection systems of WWTPs, and therefore can be considered indicators of the efficacy of depuration and disinfection treatment systems [42].

3. Results and discussion

3.1 *Preliminary data considerations*

This research intends to use (for general and systematic considerations on microbiological pollution of the whole water system in the area under consideration) information obtained from systematic analyses carried out in institutional monitoring networks according to the laws in place during 2000–2004. This period was chosen to work on a complete series of data for all the monitoring networks involved that have been available since the introduction of Italian regulation on water protection [43] (effective in the period of the study).

Since 1 January 2000, the surface water monitoring network has been reorganized, and the control system of discharges (and in particular the control of WWTP discharges) has become completely effective. Nevertheless, data referred to the marine-coastal water monitoring network are not complete, and therefore their consideration for the overall period has only an indicative value. For WWTPs, in data assessment, the private industrial treatment plant (with ozone disinfection system) was not considered, because the available data set is too small. It must be noted that disinfection systems are not activated in all WWTPs all through the year: usually they are activated during the bathing season according to Italian law (1 April to 30 September); in some cases, this applies all through the year.

To provide extensive and systematic information on the importance of biological pollution, based on the area under investigation, the following parameters have been assessed:

- *Quantitative parameters*: indicators of faecal contamination (TC, FC, FS, EC), which do not represent human pathogens, but whose specific concentrations indicate the probability of the concomitant presence of pathogenic bacteria and viruses of the human gastrointestinal stretch (these last two categories present a more difficult finding but can produce gastrointestinal infections of varying severity, also at low concentrations); for these types of parameters, the mean value and $\log_{10}$ value of the mean have been calculated; it should be noted that because of the high variability of the microbiological data, rigorous statistical criteria cannot be applied.
- *Qualitative parameters*: bacteriological gastroenteric pathogens (genus *Salmonella*), viral pathogens (genus *Enterovirus*), for which the isolation-frequency percentage has been recorded over the years.

From investigations on the presence of microbial and viral organisms in the inflow and outflow wastewaters, it has been possible to verify the efficiency of different disinfection technologies applied for the selected WWTPs. The creation of a database on water monitoring (rivers and sea) and characterization of WWTP effluents allowed the bacterial and viral impact of wastewaters from WWTPs on coastal waters to be evaluated. In the integrative study (2003–2004), the following were analysed: (1) 24 samples on the inflow wastewaters of the selected

WWTP and 51 samples on the outflow wastewater after the disinfection systems; (2) 96 samples on surface water (rivers).

3.2 *Microbiological characterization of surface waters: Synthetic Pluriennal Faecalization Index*

In the microbiological quantitative characterization (for TC, FC, FS, and EC parameters), the Synthetic Pluriennial Faecalization Index (ISPF), expressed as $\log_{10}$ of the mean value of cfu per 100 mlL of faecal bacteria over the whole period considered, was calculated and assessed; the indicator was developed from Italian technical references [44]. Table 3 lists the mean data (and $\log_{10}$ of the mean) for the microbiological parameters of surface waters from all the monitoring stations considered in the study; the parameter EC is not available for bathing and marine-coastal waters and therefore is not presented here for the integrated quality assessment. Tables 4 and 5 report data on *Salmonella* and *Enterovirus* as percentage presence.

Table 3 provides a comparison of faecal contamination (TC, FC, and FS in the upper stations of rivers with respect to the WWTPs considered), it is evident that the local contribution is not significant. Our results indicate a level of mean contamination of 10^4 for CT, 10^3 CF, and 10^2 for SF. Also, for the mean annual distribution of pathogenic bacteria and viruses in the last years, the principal source is represented by the river flow. In fresh surface water 34% of samples are positive for the *Salmonella* parameter, but the contribution from WWTPs is two to three times lower (14% at the WWTPs discharges) without considering the dilution effect (table 4).

The pathogen *Salmonella* is isolated also in marine waters and in coastal bathing waters, albeit with a lower frequency (1% and 4%, respectively). Similar considerations can apply to viruses (table 5), which on the other hand seem to have a smaller resistance in marine waters (absence of isolations in the 72 analysed samples). It must be noted that viral determinations

Table 3. Mean values of faecal indicators (ISPF) calculated from the total number of stations for 2000–2004.

		Total coliforms (UFC per 100 ml)		Faecal coliforms (UFC per 100 ml)		Faecal streptococci (UFC per 100 ml)	
Water body	No. of samples	Mean	Log mean	Mean	Log mean	Mean	Log mean
Rivers (total stations)	573	14 139	4.15	1765	3.25	280	2.45
Rivers (upper stations*)	444	13 425	4.13	1704	3.23	269	2.43
Total bathing stations	5627	248	2.4	30	1.48	4	0.55

*Upper stations are all stations of the monitoring network of rivers considered in the study which are localized before the discharges of WWTPs and all the ones in the rivers in which there are no WWTPs discharges (stations 432, 72, 65, 237, 436, 437, 217, and 222; see table 1 and figure 1).

Table 4. Presence of the pathogen *Salmonella* in the analysed samples and its percentage distribution in the environment for 2000–2004.

		Salmonella		
Water bodies	Total no. of samples	No. of samples	Presence	% positive
Total river stations	573	573	192	**34**
Total bathing stations	5627	1458	52	**4**
Total marine-coastal stations	170	170	2	**1**

Table 5. Cytopathogenic virus isolation and enterovirus identification for the whole system considered in the study: distribution of the percentage of enteroviruses for 2000–2004.

		Viruses				
Water body	Total no. of samples	No. of samples	No. of cytopathogenic viruses isolated	%	No. of enterovirus identifications	%
Total river stations	573	103	41	40	14	**14**
Total marine-coastal stations	170	72	0	0	0	**0**

on marine-coastal waters are performed in the summer when the temperature reduces the viability of enteroviruses.

3.3 *General indication on microbiological characterization of WWTPs*

In the project, nine public WWTPs were considered. For the integrative campaign, a private treatment plant with an ozone disinfection system was added (industrial plant no. 10; see figure 1). Tables 6–8 present a microbiological characterization of WWTP discharges for the period 2000–2004. The private industrial plant no. 10 is inserted only in the IN–OUT comparison relative to the integrative campaign, because the plant discharge does not directly influence the sea but is received by the Venice Lagoon catchment basin.

Table 6 reports the logarithms of the mean values (ISPF), determined for the final effluent of the WWTPs considered in the study during 2000–2004 for the different faecal indicators. Table 7 reports data concerning the presence of *Salmonella*, and table 8 reports data on the presence of enterovirus on discharges of WWTPs in the same period. In table 6, the activation period for disinfection systems is indicated. In general, the period of no disinfection corresponds to the period of the lowest or without the presence of tourists.

The cases in which the faecal indicators are lower, and *Salmonella* and enterovirus are less present, are those with peracetic acid and UV disinfection systems. This was not true in all cases with peracetic acid for *Salmonella*: with WWTP nos 3 and 5, the treatment plant was

Table 6. Logarithm of mean values (ISPF) for 2000–2004, for the different faecal indicators determined on the final effluent of WWTPs considered in the study.

WWTP (for localization, see figure 1)	Disinfection technique	Period of activation	Total coliforms (log cfu per 100 ml)	Faecal coliforms (log cfu per 100 ml)	Faecal streptococci (log cfu per 100 ml)	*Escherichia coli* (log cfu 100 ml)
1	NaOCl	1 April to 30 September	5.32	4.25	3.52	3.89
2	NaOCl	1 April to 30 September	3.91	3.21	2.77	3.05
3*	CH_3COOOH	1 January to 31 December	5.85	4.72	4.31	4.63
4	NaOCl	1 April to 30 September	5.31	4.27	3.84	3.97
5†	CH_3COOOH	1 January to 31 December	3.87	3	2.76	2.79
6	CH_3COOOH	1 April to 30 September	4.63	3.75	2.87	3.43
7	NaOCl	1 April to 30 September	4.46	3.9	3.14	3.53
8	NaOCl	1 April to 30 September	4.52	4.09	3.32	3.85
9	UV + light NaOCl	1 January to 31 December	3.99	3.09	2.22	2.18

*Activation of the peracetic disinfection system since 2002.
†Activation of the peracetic disinfection system since 2003.

Table 7. Total number of samples and percentage of *Salmonella* presence, in the years 2000–2004, determined on the final effluent of the WWTPs considered in the study.

WWTP (for localization see figure 1)	Disinfection technique	Total no. of samples	Total analysis of *Salmonella*	No. positive analysis for *Salmonella*	% presence of *Salmonella*
1	NaOCl	14	11	1	**9**
2	NaOCl	13	12	0	**0**
3	CH_3COOOH	7	7	3	**43**
4	NaOCl	15	10	3	**30**
5	CH_3COOOH	14	12	1	**8**
6	CH_3COOOH	16	15	5	**33**
7	NaOCl	13	11	2	**18**
8	NaOCl	12	9	0	**0**
9	UV + light NaOCl	20	19	0	**0**
Total		124	106	15	**14**

Table 8. Total number of analysis with positivity of cytopathogenic virus and percentage of enterovirus identification (the percentage of enterovirus identification is calculated on 'total analysis' on final effluent) for 2000–2004.

WWTP (for localization see figure 1)	Disinfection technique	Total no. of samples	Total analysis	Cytopathogenic viruses virus isolated	Enterovirus identification	% cytopathogenic isolation	% enterovirus identification
1	NaOCl	14	13	4	2	31	**15**
2	NaOCl	13	12	0	0	0	**0**
3	CH_3COOOH	7	6	1	0	17	**0**
4	NaOCl	15	12	1	1	8	**8**
5	CH_3COOOH	14	11	2	0	18	**0**
6	CH_3COOOH	16	15	1	1	7	**7**
7	NaOCl	13	12	3	1	25	**8**
8	NaOCl	12	10	0	0	0	**0**
9	UV + light NaOCl	20	15	2	1	13	**7**
Total		124	106	14	6	13	**6**

improved only in the last few years (since 2002 and 2003, respectively). For table 6, it should be noted that the mean is calculated for the whole period, and the disinfection systems are mostly activated only in the bathing season (1 April to 30 September). Data reported in tables 7 and 8, the efficacy of NaOCl on *Salmonella* and of peracetic acid on enterovirus is evident.

Our results suggest that not necessarily do the highest level of faecal indicators correspond the highest frequencies of isolation of pathogenic bacteria and viruses. Further emphasising the necessity of a review of faecal indicators suggested by the legislation directives.

3.4 *Biological parameters in the integrative samples of the study (years 2003–2004): WWTPs*

3.4.1 Bacterial indicators of faecal contamination. Figures 2 and 3 present mean data [$\log_{10}$(mean) = ISPF] produced in the integrative study during 2003–2004 for the influents and effluents of the WWTPs and the private industrial treatment plant no. 10 (ozone disinfection system).

The disinfection systems guarantee a reduction in faecal indicators, normally of two orders. The mean level of final discharge is higher in WWTPs where the total load is higher than the

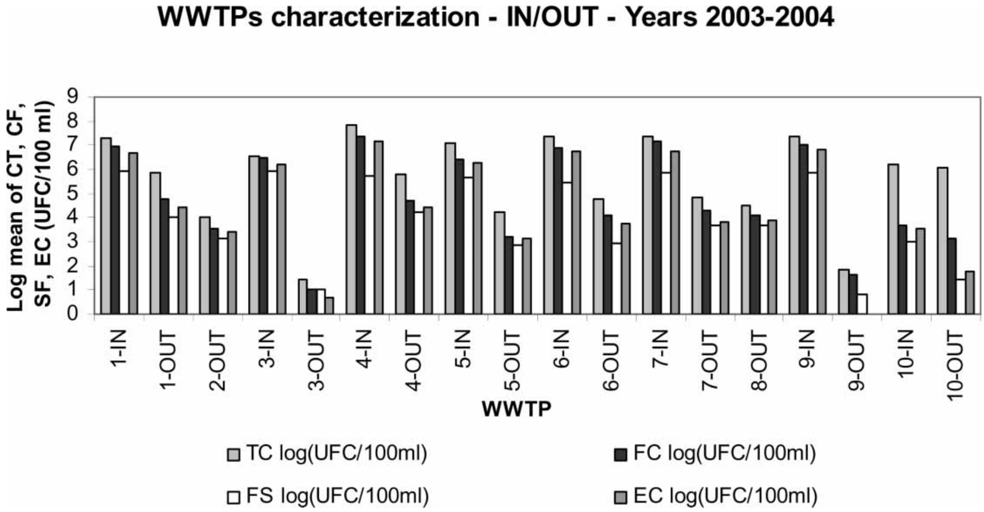

Figure 2. Comparison of logarithmic mean values (ISPF) in 2003–2004 for the different faecal indicators in the influents and effluents of WWTPs (with the disinfection systems) and private treatment plant, selected in the study. WWTPs 2 and 8 have no influent characterization. The disinfection systems are indicated in table 6.

normal project capacity of treatment for the plants (i.e. nos 1, 2, and 4). The disinfection efficacy is confirmed for *Salmonella*, but for enterovirus this depends on the type of disinfection.

Figure 4 shows the reduction in bacterial loads during the depuration processes, according to the disinfection technologies applied, during the activation period for the investigated WWTPs. The histograms refer to the mean concentration of micro-organisms assessed in the study period. The microbiological analysis found values of 10^6–10^8 (cfu per 100 ml) with a variable reduction according to the treatment shown in the graph; the UV technology appears to produce a greater reduction but less so for peracetic acid.

A relative scarce efficacy of the bacterial density abatement was observed in the WWTPs which, during the investigated period, used as disinfection system the sodium hypochlorite (NaClO); this result can probably be related to the high organic loads reported during the tourist

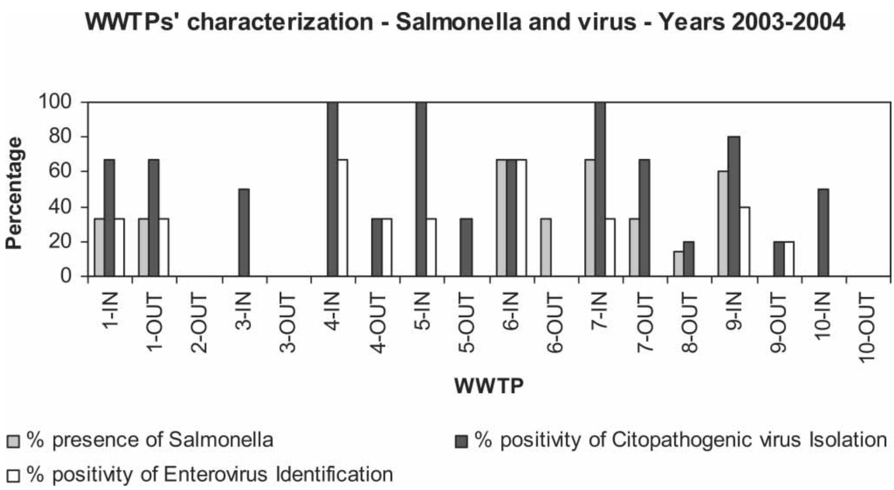

Figure 3. Percentage presence of *Salmonella* and positivity of cytopathogenic virus and enterovirus identification for the influents and effluents of public WWTPs and the private treatment plant during the campaign (2003–2004). The disinfection systems are indicated in table 6.

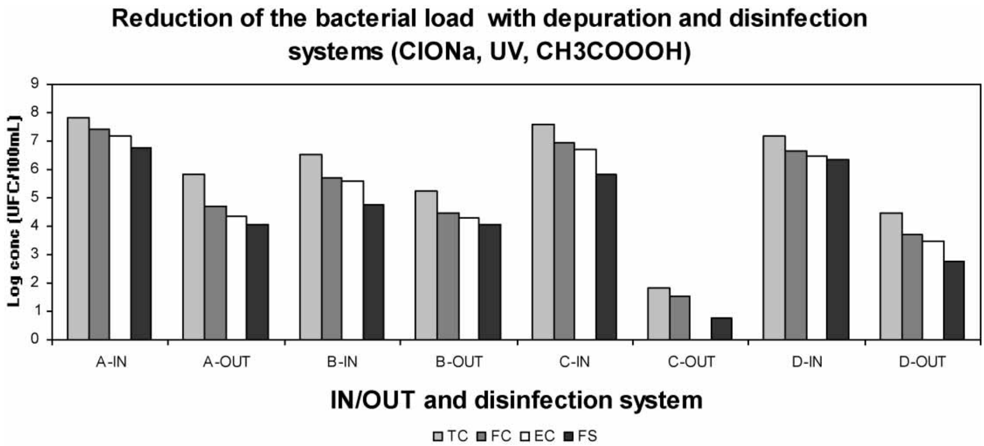

Figure 4. Reductions in bacterial loads expressed as ISPF for 2003–2004 (A with hypochloride disinfection, for 1 April to 30 September; B without hypochloride disinfection, for 1 October to 31 March; C with UV disinfection; D with peracetic acid. TC: faecal coliforms; FC: faecal coliforms; EC: *Escherichia coli*; FS: faecal steptococci.

season. UV treatment processes associated with light chlorination (WWTP no. 9) produce a remarkable reduction; this reduction is less evident for peracetic acid. In figure 4, the ozone disinfection system (plant no. 10; see figures 2 and 3) is not considered according to the few data available in comparison with other technologies.

The normal capability of faecal bacteria abatement of WWTP without disinfection is of one order (B); it must be noted that this situation also corresponds to the period with limited tourism.

3.4.2 Pathogens: *Salmonella* and viruses. During 2003–2004, *Salmonella* was present in 33% (of 24 samples) of influents and 20% (of 51 samples) of effluents in WWTPs (table 9). The samples include public WWTPs and private industrial plant no. 10, which uses an ozone disinfection system.

The results suggest a limited presence of *Salmonella* in the influents as a consequence of the local good epidemiological situation and the presence percentages according to the disinfection systems are reported in table 12.

Table 9. Presence of *Salmonella* spp. in wastewaters for 2003–2004.

WWTP	No. of positive samples	% positive samples
In	8 of 24 samples	33
Out	10 of 51 samples	20

Table 10. Presence of enteric viruses for 2003–2004.

WWTP	No. of positive samples/total no. of samples	% positive samples
In	19 of 24	79
Out	12 of 51	23

Table 11. Presence of enteroviruses for 2003–2004.

WWTP	No. of positive samples/total no. of samples	% positive samples
In	9 of 24	33
Out	5 of 51	10

Table 12. Disinfection systems and presence of Salmonella spp., enteric viruses, and enteroviruses for 2003–2004.

	% positives in effluents of WWTPs		
Disinfection systems	*Salmonella* spp.	Enteric virus	Enterovirus
CH_3CO_2OH	$0/10 = 0\%$	$1/10 = 10\%$	$0/10 = 0\%$
UV	$0/7 = 0\%$	$1/6 = 17\%$	$1/6 = 17\%$
NaClO 1 April to 30 September	$0/11 = 0\%$	$3/11 = 27\%$	$2/11 = 18\%$
Absence of disinfection 1 October to 31 March	$10/21 = 48\%$	$7/21 = 33\%$	$2/21 = 10\%$

The presence of enteric viruses and enteroviruses in the influents was 79% and 33%, respectively, and in the effluents, 23% and 10% (tables 10 and 11).

Table 12 presents the efficacies of *Salmonella* and viral reduction according to disinfection system and the results (as a percentage) of viral determination in the 2003–2004 experimental campaigns for influents and effluents of public WWTPs.

Treatment systems with peracetic acid yielded the best percentage reduction in viral loads, while the reduced action of sodium hypochlorite (NaClO) during the permitted usage period can be explained by the higher organic load, as a consequence of the presence of tourists. All disinfection systems showed a good efficacy in reducing *Salmonella*.

3.5 *Rivers: integrative samples in the study (years 2003–2004)*

This study relates to nine rivers (table 1 and figure 1) selected according to the localization of the WWTPs considered in the study for the assessment of the dilution of pollution and microbiological contamination. During the study period, it was possible to assess the seasonal nature of enteric viruses.

3.5.1 *Salmonella* spp. The presence of *Salmonella* in surface-water samples was 38% (on 96 samples) (table 13), and *Salmonella* was found in all investigated stations, albeit with different frequencies.

Table 13. Presence of *Salmonella* in all river samples for 2003–2004.

Water body	No. of positive samples	% positive samples
Rivers	36 of 96 samples	38

Table 14. Presence of enteric virus and enteroviruses in samples from rivers for 2003–2004.

Samples	Total no.	Total no. of positive enteric virus	No. of positive enterovirus	% positive enteric virus	% positive enterovirus
Rivers	96	36 of 96	14 of 96	38	15

3.5.2 Virus. Thirty-eight percent of samples tested positive for enteric viruses and 15% for enteroviruses (table 14). From these results, a restricted circulation of enteroviruses is evident.

The presence of enteric viruses was revealed in all rivers, albeit with different frequencies: in some of cases, positive results for enteroviruses were confirmed. The isolation frequency was higher during the winter, spring, and autumn (31 of 71 samples, 44%) than during the summer (7 of 21 samples, 33%): the higher temperatures and greater solar radiation greatly favour their inactivation as indicated in the literature [45]. River monitoring displayed the presence of pathogenic bacteria and viruses in the areas around the WWTPs.

3.6 *Integrated quality assessment*

From the integrated quality assessment of the coast, in many cases there is a high level of mean faecal contamination. In the comparison of results for each stretch, the most critical situation is the coastal stretch no. VIII (beaches of Ca' Roman, Sottomarina-Chioggia and Isola Verde; see table 1 for the localization of the monitoring points). The results are widely attributed to pressure from the Brenta and Adige rivers.

The analysis presented in this study gives a static representation of the territory because it does not consider hydrological and meteorological variables. Sites VI and VII (see table 1 for monitoring points), the area between Punta Sabbioni (Cavallino coast) and Pellestrina coast, are those which present the best environmental conditions according to faecal contamination indices; this situation depends on the following factors: (1) the WWTP nos 7 and 8 both have the discharge point 4 km from the coast (underwater pipes), thus being far away from the bathing-water monitoring stations, and this is in accordance with other findings in the literature [20]; (2) the lack of river mouths in the two sites VI and VII.

In situations in which the presence of *Salmonella* is not found in discharge wastewaters in the influents or effluents of WWTPs, one can see instead its presence in several monitoring points in surface waters: the livestock origin (sewage spreading) or diffuse domestic sewage presence in the area (diffuse pollution) [12]. In fact, this has been observed in 20% of samples of wastewaters and 38% of surface freshwaters (integrative study, 2003–2004). This is probably caused by livestock or human diffuse pollution, as the WWTPs contribute to the release of insignificant and discontinuous levels of pathogens.

Although this study has indicated a low circulation of enterovirus (15% of samples of surface waters and 10% of effluent of WWTPs in 2003–2004), their presence should not be underestimated because infections are also possible with very low abundance [6].

4. Conclusions

This study has produced a large database of microbial components in fresh-water, bathing and marine coastal waters in the period 1997–2004.

According to Water Framework Directive 2000/60/EC and Directive 2006/7/EC, an integrated approach for the study of water-quality control of pressure sources and assessment of intervention measures was conducted.

For the evaluation of pollution, the ISPF has been applied. For a better understanding of the environmental microbiological impact, eight homogeneous areas along the coast have been identified, through an integrated quality assessment on the matrices investigated using the DPSIR model, comparing local contributions and the contributions from rivers.

Our results display bacterial and viral reductions at nine public WWTPs with different disinfection systems (peracetic acid, UV rays, and sodium hypochloride) and at one industrial plant (ozone disinfection system). Disinfection, except for some cases, is applied only during the bathing period (1 April to 30 September): this favours the presence of moderate bacteriological contamination in waters during the autumn–winter season. On the other hand, in this period, a significant viral contamination is favoured by low temperatures.

During the period of disinfection, an evident reduction in pathogenic bacteria (*Salmonella*) has been reported, when these are present in influent sewage, and a significant reduction of viruses according to the disinfection technology used. Moreover, our results suggest that in some cases, pathogens are not present in the influents and effluents of the WWTPs but are present in river water; this means that there is some environmental contamination of a different origin, proven also by the health controls on the population.

As preliminary indications sodium hypochloride and UV rays are effective on Salmonella as is peracetc acid on viruses. Data on influents are not sufficient to outline definitive conclusions on the efficacy of disinfection technologies and microbiological circulation in the WWTPs. For the ozone disinfection systems, the available data were not sufficient to provide specific indications.

This study forms a preliminary characterization of all investigated matrices and of WWTPs with different disinfection systems. The number of samples on the WWTP inflow and outflow in the experimental activity is limited, and therefore further studies should be carried out in a new experimental campaign; moreover, *Salmonella* and enteroviruses should be quantified (i.e. not only the presence/absence).

Acknowledgements

The authors thank the two anonymous referees whose observations were particularly useful for significant modifications of the text and for the general structure of the paper, and the ARPAV Provincial Department of Venice, in particular the entire personnel of the Laboratory Service and the Territorial Service. Moreover, the authors thank Mr Lucio D'Alberto for the general review of the paper and the useful suggestions.

References

[1] V.J. Cabelli. Health effects criteria for marine recreational waters, US Environmental Protection Agency. EPA-600/1-80-031, 98 (1983).

[2] S. Glasoe, A. Christy. Coastal Urbanization and Microbial Contamination of Shellfish Growing Areas. PSAT04-09, State of Washington, Olympia, WA (2004). Available online at: http://www.psat.wa.gov/Programs/shellfish/sf_lit_review0604.pdf (accessed June 2006).

[3] M. Vazzoler, E. Aimo, L. Berti, R. Biancotto, E. Cabras, M. Ostoich, C. Rossi, C. Soccorso, A.R. Zogno. Integrated analysis of activities developed by ARPAV-Veneto Region in the marine-coastal zone: contributions to hygienic and sanitary evaluation of the bathing waters in the Province of Venice (years 1997–2001). Poster presented to the IIIrd National Workshop of Marine Sciencs 'Extreme environments and transitional areas', organized by CoNISMa, Bari (Italy), 27–29 November 2002 (2002).

[4] S.M. Goyal, C.P. Gerba, J.L. Melnick. Occurrence and distribution of bacterial indicators and pathogens in canal communities along the Texas Coast. *Appl. Environ. Microbiol.*, **34**, 139–149 (1977).

[5] N. Philip, Sewage in the marine environment: A case history from Sydney NSW. In *The State of the Marine Environment Report for Australia—Issues in the Marine Environment*, Great Barrier Reef Marine Park Authority, Townsville (1994).
[6] L.P. Zann, D. Sutton. State of the Marine Environment Report for Australia: Pollution Technical Annex 2. *Environment Australia—Coasts & Oceans Publications*, Canberra (1995). Available online at: http://www.deh.gov.au/coasts/publications/somer/annex2/ashbolt.html (accessed June 2006).
[7] K. Obiri-Danso, K. Jones. The effect of a new sewage treatment plant on faecal indicator numbers, campylobacters and bathing water compliance in Morecombe Bay. *J. Appl. Microbiol.*, **86**, 603–614 (1999).
[8] M. Griesel, P. Jagals. Faecal indicator organisms in the Renoster Spruit system of the Modder-Riet River catchment and implications for human users of the water. *Water SA*, **28**, (2002). Available online at: http://www.wrc.org.za (accessed June 2006).
[9] European Directive 2006/7/EC concerning the management of bathing water quality and repealing Directive 76/160/EEC, OJ 4/03//2006 no. *L 64*.
[10] J.B. Rose, R.M. Atlas, C.P. Gerba, M.J.R. Gilchrist, M.W. LeChevallier, M.D. Sobsey, M.V. Yates, G.H. Cassell, J.M. Tiedje. *Microbial Pollutants in our Nation's Water: Environmental and Public Health Issues*. American Society for Microbiology. Washington, DC (1999). Available online at: http://www.asm.org/ASM/files/CCPAGECONTENT/DOCFILENAME/0000005987/waterreport[1].pdf (accessed June 2006).
[11] M.D. Sobsey. Inactivation of health-related microorganisms in water by disinfection processes. *Water Science and Technology*, **21**, 179–195 (1989).
[12] A.M. Donnison, C.M. Ross. Animal and human faecal pollution in New Zealand rivers. *New Zealand Journal of Marine and Freshwater Research*, **33**, 119–128 (1999).
[13] B. O'Keefe, B.J. D'Arcy, J. Davidson, B. Barbarito, B. Clelland. Urban diffuse sources of faecal indicators. *Water Science and Technology*, **51**, 183–190 (2005). Available online at: http://www.iwaponline.com/wst/05103/wst051030183.htm (accessed June 2006).
[14] R.T. Noble, J.A. Fuhrman. Enteroviruses detected by reverse transcriptase polymerase chain reaction from the coastal waters of Santa Monica Bay, California: Low correlation to bacterial indicators. *Hydrobiologia*, **460**, 175–184 (2001).
[15] R.T. Noble, D.F. Moore, M.K. Leecaster, C.D. McGee, S.B. Weisberg. Comparison of total coliform, fecal coliform, and enterococcus bacterial indicator response for ocean recreational water quality testing. *Water Research*, **37**, 1637–1643 (2003). Available online at: http://www.ochealthinfo.com/docs/public/h2o/Water-Research-Publication-2003.pdf (accessed June 2006).
[16] M.A. Moriñingo, M.A. Muñoz, R. Cornax, E. Marinez-Manzanares, J.J. Borrego. Presence of indicators and *Salmonella* in natural waters affected by outfall wastewater discharges. *Water Sci. Technol.*, **25**, 1–8 (1992).
[17] M. Pommepuy, J.F. Guillaud, E. Dupray, A. Derrien, F. Le Guyadee, M. Cormier. Enteric bacteria survival factors. *Water Sci. Technol.*, **25**, 93–103 (1992).
[18] C.L. Meays, K. Broersma, R. Nordin, A. Mazumder. Source tracking fecal bacteria in water: a critical review of current methods. *J. Environ. Manage.*, **73**, 71–79 (2004).
[19] D.L. Craig, H.J. Fallowfield, N.J. Cromar. Comparison of decay rates of faecal indicator organisms in recreational coastal water and sediment. *Water Supply*, **2**, 131–138 (2002). Available online at: http://www.iwaponline.com/ws/00203/ws002030131.htm (accessed June 2006).
[20] G.S. Grohmann, N.J. Ashbolt, M. Genova, G. Logan, P. Cox, C.S.W. Kueh. Detection of viruses in coastal and river water systems in Sydney Australia. *Water Sci. Technol.*, **27**(3–4), 457–461 (1993).
[21] New Zealand Ministry for the Environment. Freshwater Microbiology Research Programme Report—Pathogen Occurrence and Human Health Risk Assessment Analysis (2002). Available online at: http://www.mfe.govt.nz/publications/water/freshwater-microbiology-nov02/freshwater-microbiology-main-nov02.pdf (accessed June 2006).
[22] L.W. Sinton, A.M. Donnison, C.M. Hastie. Faecal streptococci as faecal pollution indicators: a review. Part II: Sanitary significance, survival and use. *New Zeal. J. Mar. Freshw. Res.*, **27**, 117–137 (1993). Available online at: http://www.rsnz.org/publish/nzjmfr/1993/10.php (accessed June 2006).
[23] Italian Decree 8/06/1982, no. 470. Bathing waters. *Italian O. J. no. 203 26/07/1982*.
[24] European Directive no. 76/160/EEC 8/12/1975 on bathing waters, *EC OJ no. L 31 of 5/02/1976*.
[25] European Directive 2000/60/EC of 23/10/2000, Water Framework Directive, *EC OJ no. L 327 of 22/12/2000*.
[26] J. Bartram, G. Rees. Monitoring Bathing Waters, E. & FN Spon, London (2000).
[27] ISTAT. Population Census. (2001).
[28] European Environment Agecy. Europe's Environment. The Second Assessment, Copenhagen (1998).
[29] European Environment Agency. Guidelines for data collection for the Dobris +3 report, EEA Technical report, 1996/1, Copenhagen (1996).
[30] J.J. Kessler, M. Van Drop. Structural adjustment and the environment: the need for an analytical methodology. *Ecol. Econ.*, **27**, 267–281 (1998).
[31] A. Barrera-Roldan, A. Saldivar-Valdes. Proposal and application of a Sustainable Development Index. *Ecol. Indic.*, **2**, 251–256 (2002).
[32] R.E. Bowen, C. Riley. Socio-economic indicators and integrated coastal management. *Ocean Coast. Manage.*, **46**, 299–312 (2003).

[33] World Bank. *Integrated Coastal Management. Coastal and Marine Management: Key Topics*. The World Bank Group, Washington, DC (2002). Available online at: http://lnweb18.worldbank.org/ESSD/essdext.nsf/42ByDocName/KeyTopicsIntegratedCoastalManagement (accessed June 2006).
[34] X. Xue, H. Hong, A.T. Charles. Cumulative environmental impacts and integrated coastal management: the case of Xiamen, China. *J. Environ. Manage.*, **71**, 271–283 (2004).
[35] Italian National Health Institute ISTISAN. Analytical methods for drinking water. *Report 97/8* (1997). Available online at: http://www.iss.it/publ/serie.php?id=5 (accessed June 2006).
[36] APAT-Italian National Environmental Protection Agency. Analytical methods for water, **3**, 29/2003, Rome (2003). Available online at: http://www.apat.gov.it/site/it-IT/APAT/Pubblicazioni/Manuali_e_linee_guida/ (accessed June 2006).
[37] Italian National Health Institute ISTISAN. *Analytical Methods for Drinking Water. Report 00/14 Pt.2* (2000). Available online at: http://www.iss.it/publ/serie.php?id=5 (accessed June 2006).
[38] US-EPA. Drinking Water Criteria Document for Viruses, Draft PB86-118270, National Technical Information Service, USEPA, Springfield, VA (1985).
[39] E.L. Elliot, R.R. Colwell. Indicator organisms for estuarine and marine waters. *FEMS Microbiol. Rev.*, **32**, 61–79 (1985).
[40] Italian Decree 14/05/1988 no. 155. Changes to decree 8/06/1982 no. 470 on bathing waters. *Italian OJ 16/05/1988 no. 113*.
[41] Italian Decree 2/02/2001 no. 31. Acknowledgement of Directive 98/83/CE—Quality of waters for human consumption *Supplement of the Italian OJ 3/03/2001 no. 52*.
[42] ARPAM–Marche Regional Environmental Protection Agency. Virus in the environment: viral investigations on surface waters and sediments in Marche region. ARPAM (2002).
[43] Italian Decree 11/05/1999, no. 152. Water protection, *Supplement of the Italian O.J. no. 246 20/10/2000*.
[44] APAT—Italian National Environmental Protection Agency. Biological indicators for marine-coastal waters, National Thematic Centre on Internal and Marine-Coastal Waters, AIM_T_RAP_03_15 (2004). Available online at: http://www.apat.gov.it/site/it-IT/APAT/Pubblicazioni/Manuali_e_linee_guida/ (accessed June 2006).
[45] F.A. Aulicino. Virus and bacteriofagi in drinking waters: meaning and detection methods. In *Drinking Waters*, Pitagora Editrice, Bologna (1995).