Assessment, control, and prevention of microbiological and chemical hazards in seasonal swimming pools of the Versilia district (Tuscany, central Italy)

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ABSTRACT

Although in Europe the quality of swimming pools (SPs) is dictated by regulations, microbiological and chemical hazards are described in the literature. Environmental bacteria or toxic disinfection by-product (DBP) compounds may indeed be recovered in waters even after disinfection. We evaluated the water quality from 26 outdoor seasonal SPs of the Versilia district, according to requirements of Regional Decree 54R/2015. In spring 2017, supply and reinstatement waters were collected after shock hyperchlorination (10 mg/L) while in summertime, a second sampling of waters before entering the pools, as well as in the pools, was performed after SPs were open to the public. In all samples, microbiological and chemical parameters were determined as defined by Directive 98/83/EC and the Italian Health Ministry. Microbiological data were within suggested limits. The first chemical analyses showed that in 35% of the feeding-pool seawater samples, the halogenated organic compounds were higher than the maximum permissible concentrations (30 μg/L). Pool waters were then dechlorinated and re-treated with hydrogen peroxide (10 mg/L) to ensure the abatement of DBPs (from 164 ± 107 to 0.9 ± 0.8 μg/L; $p = 0.002$). Results highlighted the need of self-controlled procedures for the SPs waters to prevent waterborne diseases and suggested hydrogen peroxide as the most appropriate disinfection method.

Key words | disinfection procedures, microbiological and chemical hazards, swimming pools, water sampling and analysis

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INTRODUCTION

European and Italian guidelines for health and safety in swimming pools (SPs) report that SPs are defined as systems equipped for bathing, recreational, training, sporting, and therapeutic activities, which are carried out in the water (Ministry of Health 2003; Health & Safety Executive 2018) and classified as public, private, condominium SPs and indoor, outdoor, and mixed SPs, which can be supplied with fresh, sea, and thermal waters (British Standards Institution 2014). They can be located in public areas, hotels, spas, and private houses, where people can go for sport, recreation, relaxation, and to socialize (Thomas & Murray 2008; Barna & Kádár 2012).

To prevent waterborne diseases and to inactivate waterborne pathogens, SP waters are always subjected to disinfection with several chemical disinfectants, such as chlorine, chloramine, chlorine dioxide, and ozone, although chlorine is the most commonly used substance. Therefore, disinfection procedures are applied to protect the exposed

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population against waterborne infections (Chowdhury *et al.* 2014).

Although the most common related risks of bathing activities are derived from injuries and trauma, pathogenic microorganisms, such as bacteria, viruses, and protozoa, may colonize SP plants. Recent studies (Liguori *et al.* 2007; Valeriani *et al.* 2012) have reported bacterial contaminations by non-fecal origin microorganisms (*Pseudomonas* spp., *Staphylococcus aureus*, *Legionella* spp., *Mycobacterium* spp.). Staphylococci are common skin organisms, which may be found in SP waters and are considered as a risk indicator for skin and eye diseases. *Pseudomonas aeruginosa* can also grow in several environmental conditions, such as those of SPs, carried by swimmers or due to the lack of appropriate water disinfection procedures. Fecal-originated bacteria, e.g., enterococci, coliforms, *Escherichia coli*, can colonize SP waters and be introduced by swimmers' bodies, organic fluids, or accidental fecal releases (Keuten *et al.* 2012; Zhan *et al.* 2014).

To prevent outbreaks of waterborne diseases, SP waters are continuously disinfected, although SP owners may not be aware of the formation of disinfection by-products (DBPs) in water, such as haloacetic acids (HAAs) and trihalomethanes (THMs) (United States Environmental Protection Agency 2006; Liu & Zhang 2014).

Worldwide studies have reported that DBP concentrations in SP waters higher than 150 μg/L favor the probability of being taken up through inhalation, ingestion, and dermal absorption (Lee *et al.* 2009; Tang 2011; Xiao *et al.* 2012), increasing the potential health risks of exposure for SP users. Hundreds of mutagenic and carcinogenic DBPs have, indeed, been identified in water (Richardson *et al.* 2007; Zhai *et al.* 2014). Overall, the presence of DBPs in SP waters may be induced by organic precursors, e.g., human sweat, urine, lotions, sun screams, cosmetics and soap residue, high temperatures, and disinfectant levels in water (Richardson *et al.* 2010; Hang *et al.* 2016).

Regional Decree 54R/2015 (Tuscany Region 2015), issued in 2015 by the Tuscan (central Italy) health authorities, provides indications for preventing and controlling SP water hazards, highlighting the importance of the evaluation of physical-chemical and microbiological indicators, although in 2003 the Italian Health Ministry had already dictated the health requirements of SP waters (Linguanti *et al.* 2018).

The purpose of this work was to monitor and assess the water quality of outdoor seasonal SPs from bathing establishments in the Versilia district (western Tuscany, central Italy), according to the requirements of the Regional Decree 54R/2015. In this study, institutional laboratories carried out environmental analyses with the aim of identifying any control critical points linked to the presence of microbiological and chemical hazards in SP waters. In this way, it is possible to manage the water risk in seasonal bathing establishments.

METHODS

Setting, disinfection methods, and statistical analysis

In spring (May–June) and summer (July–August) 2017, 26 seasonal SPs' waters were selected to evaluate and monitor the risks related to the SPs' activities. For each SP, a self-controlled plan was drawn up according to the requirements of the Italian Health Ministry. Self-controlled plans included the main regulatory references and directives in the field of drinking water safety procedures (European Parliament 1998), SPs' identification data, risk assessment plans for water pools, and self-controlled procedures for the SP activities.

All investigated SPs were equipped with diatomaceous earth filters, pumping systems, chlorinator devices, control unit systems for pH and free chlorine detection, compensation pool with overflow channels and a main pool (surface area from 150 to 320 m^2 and volume from 200 to 410 m^3).

All SPs were supplied with pre-treated seawater, whereas reinstatement was carried out with drinking or domestic well water. Seawater was disinfected with sodium hypochlorite (10 mg/L). After 2 weeks, the total chlorine concentration was adjusted at 1 mg/L.

In the case of the presence of chemical hazards, pool water was dechlorinated by activated carbon filters and re-treated with hydrogen peroxide (10 mg/L) to ensure the absence of DBPs. After 2 weeks, water was again treated with sodium hypochlorite allowing the presence of a final total chlorine concentration of 1 mg/L.

The Kolmogorov–Smirnov test was performed to verify the normality of distributions. For each SP, Kruskall–Wallis

and Dunn tests were used to compare the DBP concentrations detected after the shock disinfections with sodium hypochlorite and hydrogen peroxide. Power tests were performed to estimate the sample sizes. The 1-beta values of the significant variables were >0.8, proving that the sample sizes were acceptable. SPSS Version 17.0.1 (IBM Corp., Armonk, NY, USA) was used for the statistical analysis.

First water sampling and microbiological and chemical analysis

One month before the opening of SPs (after the shock treatment) (May–June 2017), the first sampling of seawater and reinstatement water (drinking or domestic wells) was carried out. The sampling plan was scheduled as described by ISO 19458:2006 and ISO 5667-1:2006 (International Organization for Standardization 2006a, 2006b) for microbiological and chemical sampling, respectively. Each sea and well water sample was subjected to a chemical, chemical-physical, and microbiological sampling to determine the parameters reported in Table 1. The detected values were compared with the maximum permissible concentrations provided by the European Directive 98/83/EC (European Parliament 1998).

The microbiological analyses were carried out for detection of the total microbial counts (TMCs) at 22 and 37 °C (International Organization for Standardization 2001) in two 1 mL aliquots using Plate Count Agar (Oxoid Ltd, Basingstoke, Hampshire, UK) as medium. Coliform bacteria, *E. coli* (International Organization for Standardization 2012), and fecal streptococci (International Organization for Standardization 2000) were enumerated by filtering 100 mL aliquots using Colilert 100 test (Idexx, USA) and Slanetz Bartley Agar (Biolife, Italy), respectively. *Pseudomomas aeruginosa* (International Organization for Standardization 2006c) and *Staphylococcus aureus* (Superior Institute of Health 2007) were searched for in a 250 mL sample using CN Pseudomonas Agar (Biolife, Italy) and Mannitol Salt Agar (Biolife, Italy). *Clostridium perfringens* (Superior Institute of Health 2007) was enumerated by filtering one 100 mL aliquot using Sulfite Polymyxin Sulfadiazine (SPS) Agar (Biolife, Italy). Filtration was performed through a 0.45 μm membrane (Nalgene, USA), which was layered on the respective culture media before being incubated at the proper temperature and for the requested period.

Table 1 | Microbiological and chemical parameters determined in the water supplies (sea and reinstatement water) after the first sampling

	Limits (European Directive 98/83/EC)
Microbiological parameters	
Total microbial counts at 22 °C	Constant values
Total microbial counts at 37 °C	Constant values
Clostridium perfringens	Absent in 100 mL
Coliform bacteria	Absent in 100 mL
Escherichia coli	Absent in 100 mL
Fecal streptococci	Absent in 100 mL
Pseudomonas aeruginosa	Absent in 250 mL
Staphylococcus aureus	Absent in 250 mL
Chemical parameters	
Ammonium	0.5 mg/L
Arsenic	10 μg/L
Chloride	250 mg/L
Halogenated organic compounds	30 μg/L
Iron	200 μg/L
Manganese	50 μg/L
Nitrate	50 mg/L
Nitrite	0.5 mg/L
Sodium	200 mg/L
Sulfate	250 mg/L

The pH and the electrical conductivity (in μS/cm) were determined with a multi-probe portable instrument PC70 while turbidimetry was carried out with a AL250T-IR (0.001–1,000 NTU (nephelometric turbidity units)) and the values were then converted to SiO_2 and expressed in mg/L. The analytical error was <5%. Ammonium (Nessler method) and nitrite (Hach-Nitriver 3 method) were measured by molecular spectrophotometry using a HACH DR2100, with a detection limit of 0.01 and 0.001 mg/L, respectively, and an analytical error of <3%. Nitrate, chloride, sulfate, and sodium were analyzed by ion chromatography with Metrohm 761 and Metrohm 861 chromatographers, respectively. The detection limits were 0.1 mg/L for nitrate and sulfate and 0.5 mg/L for chloride and sodium. The analytical error was <5%. Arsenic, iron, and manganese (detection limits of 0.1, 5, and 0.1 μg/L,

respectively) were determined by ICP-MS (inductively coupled plasma-mass spectrometry) according to EPA 6020B (United States Environmental Protection Agency 2014a) with an analytical error <10%. Samples for the determination of the solutes were filtered at 0.45 μm.

The halogenated organic compounds were analyzed by GC-MS (gas chromatography-mass spectrometry) equipped with a purge and trap device following EPA 5030C and the EPA 8260D (United States Environmental Protection Agency 2003, 2014b). The detection limits were 0.1 μg/L for tetra-chloro-ethylene and tri-chloro-ethylene and 0.01 μg/L for chloroform, bromoform, dichloro-bromo-methane, chloro-bromo-methane, and 1,2 dichloro-ethane. The analytical error was <10%.

Second water sampling and microbiological and chemical analysis

In July–August 2017, i.e., two months after the SPs were open, waters were newly collected, as follows: before the water entered the pool and inside the pool. Microbiological and chemical sampling was performed as described above.

At both sampling points waters were collected to detect the parameters shown in Table 2. The detected values were compared with limits provided by the Italian Ministry of Health (Ministry of Health 2003).

Chemical and microbiological analyses were performed as described above, while temperature, free and combined chlorine were measured during the sampling procedures, using a digital thermometer Pt1000 (VWR, Italy) and a Visocolor HE Chlorine 2 (Macherey-Nagel, Germany).

RESULTS

First sampling

The microbiological parameters were below the limits recommended by Council Directive 98/83/EC in all the 26 SPs analyzed. Therefore, in all seawater samples, we detected total microbial counts at 22 and 37 °C in the range between 1 and 44 CFU/mL. On the other hand, in the reinstatement water samples, at 22 and 37 °C the total microbial counts were between 1 and 74 CFU/mL. In accordance with the same regulations, coliform bacteria, *E. coli, Pseudomonas aeruginosa*, and *Staphylococcus aureus* were not isolated. During the first sampling almost all chemical values were within the recommended limits, with the exception of the halogenated organic compounds, which were higher than 30 μg/L in nine out of 26 (35%) seawater samples (Table 3).

After dechlorination and treatment with hydrogen peroxide in the nine SPs where high contents of halogenated organic compounds were found, a new microbiological and chemical seawater sampling was carried out to evaluate whether the water quality was improved. The microbiological parameters were found to be within the recommended limits, and an abrupt decrease of the contents of the halogenated organic compounds was detected in all nine seawater samples: from 164 ± 107 μg/L to 0.9 ± 0.8 μg/L ($p = 0.002$) (Figure 1).

Table 2 | Microbiological, chemical, and chemical-physical parameters determined during the second sampling in waters collected before the water entered the pool and inside the pool

	Limits for the entrance of pool (Ministry of Health 2003)	Limits for pool (Ministry of Health 2003)
Microbiological parameters		
Total microbial counts at 22 °C	≤100 CFU/mL	≤200 CFU/mL
Total microbial counts at 37 °C	≤10 CFU/mL	≤100 CFU/mL
Escherichia coli	Absent in 100 mL	Absent in 100 mL
Fecal streptococci	Absent in 100 mL	Absent in 100 mL
Pseudomonas aeruginosa	Absent in 100 mL	Absent in 100 mL
Staphylococcus aureus	Absent in 100 mL	Absent in 100 mL
Chemical parameters		
Ammonium	0.5 mg/L	0.5 mg/L
Chloride	250 mg/L	250 mg/L
Nitrate	50 mg/L	≤20 mg/L
Nitrite	0.5 mg/L	0.5 mg/L
Total chlorine	0.6–2 mg/LCl_2	0.7–2 mg/LCl_2
Chemical-physical parameters		
pH	6.5–7.5	6.5–7.5
Temperature	18–30 °C	18–30 °C

Table 3 | Results of total microbial counts at 22 and 37 °C and halogenated organic compounds determined in sea and in reinstatement water samples in the 26 SPs analyzed

First sampling

	Sea			Reinstatement		
SPs	**TMCs at 22 °C (CFU/mL)**	**TMCs at 37 °C (CFU/mL)**	**Halogenated organic compounds (μg/L)**	**TMCs at 22 °C (CFU/mL)**	**TMCs at 37 °C (CFU/mL)**	**Halogenated organic compounds (μg/L)**
SP1	2	2	397	1	1	<30
SP2	2	2	<30	1	1	<30
SP3	1	1	<30	2	1	<30
SP4	3	3	114	2	2	<30
SP5	2	2	123	2	2	<30
SP6	3	2	119	2	2	<30
SP7	14	12	<30	34	32	<30
SP8	2	2	<30	2	2	<30
SP9	44	40	60	3	2	<30
SP10	2	2	<30	2	2	<30
SP11	5	5	92	7	6	<30
SP12	1	1	264	1	1	<30
SP13	1	1	105	1	1	<30
SP14	2	2	<30	1	1	<30
SP15	1	1	<30	50	45	<30
SP16	1	1	<30	1	1	<30
SP17	3	2	<30	25	25	<30
SP18	1	1	199	74	70	<30
SP19	2	2	<30	1	1	<30
SP20	3	2	<30	1	1	<30
SP21	1	1	<30	1	1	<30
SP22	4	4	<30	62	54	<30
SP23	15	10	<30	9	7	<30
SP24	1	1	<30	1	1	<30
SP25	5	5	<30	1	1	<30
SP26	2	2	<30	2	2	<30

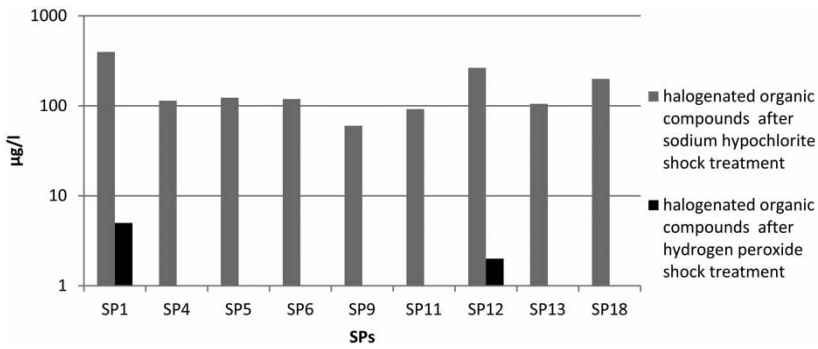

Figure 1 | Comparison between the concentrations of the halogenated organic compounds (in μg/L) detected in seawater samples after shock disinfections performed with sodium hypochlorite (gray bars) and hydrogen peroxide (black bars).

Table 4 | Results of total microbial counts at 22 and 37 °C, total chlorine, pH, and temperature detected in water samples collected from the pool and at the entrance of the pool in the 26 SPs analyzed

Second sampling

	Pool					Entrance of pool				
SPs	TMCs at 22 °C (CFU/mL)	TMCs at 37 °C (CFU/mL)	Total chlorine (mg/L)	pH	Temperature (°C)	TMCs at 22 °C (CFU/mL)	TMCs at 37 °C (CFU/mL)	Total chlorine (mg/L)	pH	Temperature (°C)
SP1	2	1	1.25	6.8	27.6	2	2	1.3	6.7	27.5
SP2	1	1	1.45	6.6	28.9	15	6	1.5	6.6	28.7
SP3	2	2	1.4	6.9	27.4	6	2	1.4	7.1	27
SP4	3	3	1.5	7.0	26.8	8	5	1.35	7.2	23.8
SP5	5	5	1.5	6.8	26.5	5	6	1.25	6.8	23.1
SP6	2	2	1.4	7.0	26.5	3	2	1.25	6.9	23.9
SP7	2	2	1.5	7.0	27.2	2	2	1.35	7.2	24.3
SP8	4	2	1.2	7.0	29.2	5	2	1.25	7.1	28.9
SP9	7	5	1.3	6.6	28.6	4	1	1.2	6.8	28.5
SP10	2	2	1.4	6.9	28.3	4	3	1.4	6.7	28.1
SP11	2	1	1.4	7.1	28.5	1	1	1.4	6.8	28.8
SP12	4	3	1.1	7.0	26.4	3	3	1.35	7	24
SP13	2	1	1.25	6.8	27	2	2	1.3	6.8	26.8
SP14	1	1	1.5	6.8	29.1	1	1	1.5	6.7	28.4
SP15	3	1	1.5	7.2	29.2	1	1	1.5	7.2	29
SP16	4	1	1	7.0	29	3	2	1.05	6.8	28.7
SP17	1	1	1.5	7.1	28.8	1	1	1.5	7	28.5
SP18	2	2	1.45	6.8	28.7	2	2	1.5	6.8	28.8
SP19	1	1	1.5	6.8	28.5	1	1	1.15	7	28.4
SP20	3	1	1.2	6.9	27	2	1	1.2	7.2	26
SP21	3	3	1.25	7.0	28.3	7	4	1.35	7	28.6
SP22	1	1	1.4	6.9	28.4	1	1	1.4	6.9	28.4
SP23	6	3	1.05	6.7	28.5	3	2	1.45	6.8	28.3
SP24	1	1	1	7.1	28.4	9	5	1.15	7	28.7
SP25	6	2	1	6.9	27.2	3	2	1.2	7.2	27
SP26	4	4	1.25	7.0	27.3	3	3	1.4	6.8	27.2

Second sampling

The microbiological and chemical data of the waters from both the pools and those entering the pools measured in July–August 2017 were below the maximum permissible concentrations, indicating a good quality of the waters (Table 4). In both sampling points the total microbial counts at 22 and 37 °C were from 1 to 15 CFU/mL and from 1 to 6 CFU/mL, respectively. No pathogen bacteria grew during the microbiological laboratory tests. Mean values of total chlorine were 1.32 ± 0.18 and 1.33 ± 0.13 mg/L in the pool water and that entering the pool, respectively. All water samples had pH values ranging from 6.6 to 7.2, while the temperature was between 24 and 29.2 °C.

DISCUSSION

Several studies assert the importance of chemical and microbial quality management in drinking water, natural

mineral water, and seawater, mostly in bathing establishments. Chemical and physical treatments, such as desalination, filtration, disinfection, may allow the improvement of different types of water (Fard *et al.* 2015; Karbasdehi *et al.* 2017, 2018; Soleimani *et al.* 2017; Totaro *et al.* 2018).

Despite the fact that disinfection procedures are a critical process for controlling waterborne infections and chlorination activities are the most used interventions during the last years in worldwide SP plants, DBPs are the undesired consequences of disinfection, mostly for chlorination procedures (Uysal *et al.* 2017; Salas *et al.* 2017). Several factors may induce the formation of DBPs in pool waters, including the type of disinfectant, filling water, and numbers of swimmers (Villanueva *et al.* 2015). These complex molecules may be incorporated through different routes, such as skin absorption, ingestion, inhalation, and exposure evaluation is of pivotal importance in epidemiological work, particularly for long-latency cancer diseases (France's Agency for Food Environmental & Occupational Health & Safety 2012).

In particular, our data demonstrated that shock treatments with sodium hypochlorite are not fully effective as halogenated organic compounds were recovered in seasonal SPs waters. The management of water quality in SPs needs further disinfection methods to avoid the presence of toxic chemical contaminants (Skibinski *et al.* 2018). Active carbon filtration can be used to remove chlorination compounds from water after the shock treatment, whereas more innovative technologies, such as reverse osmosis and nanofiltration, may be applied to remove haloacetic acids from SP water (Tang 2011). Nevertheless, to the best of our knowledge, no specific studies have been carried out to verify the effectiveness of hydrogen peroxide for SP water disinfection. Hydrogen peroxide is a strong oxidizing agent that oxidizes microorganisms' enzymatic systems, releasing free oxygen atoms. In water, hydrogen peroxide is bactericidal at a 3% solution and a sterilant at 6% in 6 h. It is more powerful than sodium hypochlorite and more stable at high temperatures and pH when compared to chlorine-based disinfectants. Furthermore, it is non-toxic to humans and the environment. It is tasteless and is not mutagenic or carcinogenic. Hydrogen peroxide decomposes rapidly in different environmental compartments, due to biotic degradation (microbial catalase and peroxidase enzymes) and abiotic degradation (transition metal, heavy metal, oxidation or reduction reactions, with organic compounds or inorganic substances) (Casini *et al.* 2017). Consequently, H_2O_2 can be regarded as a valid alternative during disinfection procedures.

CONCLUSIONS

Our data evidenced that hydrogen peroxide prevented the formation of DBPs, the concentrations of halogenated organic compounds being two orders of magnitude less than those recorded after the sodium hypochlorite treatment. This study highlighted for the first time the application of hydrogen peroxide for the shock disinfection in SP waters, and it could be included in specific self-controlled plans of SPs. Consequently, every bathing facility with a SP may better ensure the safety of swimmers.

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