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Untouchability of natural spa waters: Perspectives for treatments within a personalized water safety plan

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ABSTRACT

Natural SPA waters and their environments were known since ancient times and used for health or recreational purposes in different societies, worldwide. The composition and uses of these spring waters may not allow standard disinfection in pools, representing a challenge for hygiene management. Several safety and quality procedures were proposed, but a systematic approach is still needed. Here, we focus on alternative strategies to provide hints for developing a sustainable Water Safety Plan, based on intrinsic water properties and photocatalytic materials. The antimicrobial activity of four different SPA waters with high mineral content and one drinkable spring water with a low mineral content, was assessed and then tested for the additional bactericidal activity of Titanium Dioxide (TiO_2) nanomaterials and/or light exposure at different wavelengths (200–635 nm). A native antibacterial activity was observed in all high mineral content waters, with a CFU reduction of 75–80%. The bactericidal action of TiO_2 showed an additional incremental effect, with a reduction of over 99% within 2–5 h. Interestingly, the antibacterial photocatalytic effect was detected also in the visible light range, with a possible pick around 450–455 nm, blue-light. Based on observed results, we propose a model for developing a water safety plan, considering water properties and bather exposure. This candidate approach is personalized on water composition and pool use, trying to avoid chemical disinfectants. Photocatalytic nanotechnologies represent one of the promising alternative treatments and can provide novel perspectives for a sustainable managing of natural SPA water hygiene.

1. Introduction

The use of natural SPA springs for recreational purposes or wellness applications is known since ancient times and these waters are available worldwide. Recently, an increase in SPA economy and wellness tourism is reported in several countries, with an intensification in Europe and in the Asiatic-Pacific area (Dryglas and Salamaga, 2018; Han et al., 2017; Valeriani et al., 2018a; McCarthy, 2017; Mavridou et al., 2014). Although few outbreaks were associated to natural SPA pools, the exposure to these waters and related environments is involving a large number of people of different ages and health conditions, posing further concerns for public health (Mavridou et al., 2018; Leoni et al., 2018; Valeriani et al., 2018a; Guida et al., 2016; WHO, 2006). The natural spring waters are considered "untouchable", due to their specific composition that should be preserved in order to maintain the claimed health benefits during balneotherapy or other authorized treatments (Sevillano et al., 2018; Morer et al., 2017). The question of the "untouchability" of SPA natural waters and their incompatibility with traditional disinfection processes were addressed in different guidelines and regulations (Tunstall, 1853; WHO, 2006; Directive 2009/54/EC, n.d.; Valeriani et al., 2018a). Indeed, in compliance with the provisions laid down in Article 4 of Directive 2009/54/EC, n.d., natural spring mineral waters cannot undergo any treatment, other than removal of unstable or undesirable elements, such as iron and arsenic in drinkable oligo-mineral waters. Moreover, the addition of bacteriostatic elements or other treatments, likely to change the abiotic and biotic factors of the natural mineral water, should be prohibited, too (Directive 2009/54/EC, n.d.). Thus, the spring water composition should remain stable within the limits of natural fluctuation and recognizable by the following two classifications: A) mineral waters can be classified in 4 groups (i-iv) based on their total salt content after evaporation (dry residues at 180 °C, mg/l): i) a very low mineral content (dry residues < 50 mg/l); ii) waters low in mineral content (dry residues > 50 and < 500 mg/l); iii) waters with a medium mineral content (dry residues > 500 and < 1500 mg/l); iv) waters rich in minerals (dry residues > 1500 mg/l) (Directive 2009/54/EC, n.d.). B) mineral waters

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can be classified according to their predominant ionic composition: bicarbonate water (bicarbonate content > 600 mg/l); sulfate water (sulfate content > 200 mg/l); chlorinated water (chloride content > 200 mg/l); calcium water (calcium content > 150 mg/l); magnesium water (magnesium > 50 mg/l); fluoride water (fluoride > 1 mg/l); ferrous water (bivalent iron content > 1 mg/l); Acidic (Free carbon dioxide content > 250 mg/l) and sodium water (sodium > 200 mg/l) (Directive 2009/54/EC, n.d.).

The management of SPA thermal water facilities is, consequently, a continuous compromise between reducing the possible contamination of the waters and maintaining their original structure (Tartanson et al., 2014; Valeriani et al., 2018b, 2018c). In order to offer the most sustainable solutions for SPA water pool facilities, the research in this field, recently moved towards green strategies, trying to avoid the addition of chemicals, implementing the natural antibacterial properties and preserving their original composition (Valeriani et al., 2018b; Valeriani et al., 2017b). Innovative approaches comprise nanotechnologies, photocatalytic systems, advanced filtration or other physical treatments (Barbot and Moulin, 2008; Wang et al., 2018; Valeriani et al., 2018a). Several strategies were considered to exploit the properties of nanotechnological materials, related to their ability to amplify the active and available surface for disinfection. Photocatalytic compounds, such as zinc oxide (ZnO) or titanium dioxide (TiO_2), in the presence of light, air and water, can generate reactive oxygen species with antimicrobial capabilities (Li et al., 2008; Ibrahim and Asal, 2017; Jeon et al., 2016). A promising approach is based on the combination of different technologies into novel integrated strategies (Valeriani et al., 2018b; Tartason et al., 2014).

Here, we report laboratory testing of antibacterial natural properties in different SPA waters and the impact of photocatalytic nanotechnologies based on TiO_2 photocatalysis (NP-TiO_2). The whole of the acquired observations was used to propose a model for developing a personalized Water Safety Plan (WSP) for SPA pool facilities.

2. Material and methods

2.1. Study design

Fig. 1 shows the flow chart containing the key points of the experimental study. A bacteriostatic activity test was performed on several natural thermal waters (W_1-W_4) with different abiotic components. The evaluation of the antimicrobial activity after TiO_2 treatment (T) was assayed on a low mineral water (BLM) and on a high mineral content thermal water (W_3). Each experiment was performed with and without TiO_2 treatment (NT) at least in duplicate.

2.2. Chemical-physical analysis of natural mineral water

Within a survey of Italian SPA waters, five thermal springs with significant differences in chemical composition have been selected based on different composition, as shown in Table 1 (Valeriani et al., 2018b). The thermal water samples were collected in sterile glass bottles of 1 L, filled and immediately hermetically sealed to prevent release of gases. During the sampling activities, temperature, pH and H_2S concentration were determined. A commercially available bottled mineral water (BNM) has been included in the experiments as a control spring water without H_2S and with a low salt content (fixed residue at 180 °C = 0.3 g/l; pH = 7.2) and microbiologically and chemically checked for drinking uses. Chemical composition was determined by different analytical procedures and reported in Table 1. Sodium was quantified by an official method based on the inductively coupled plasma optical emission spectrometry (USEPA, 2003); bromide and iodide were quantified by an official method based on the ionic chromatography (USEPA, 2003); H_2S was quantified by an official method based on iodometric titration (USEPA, 2003); pH was measured by the official potentiometric method; fixed residue was determined by the official gravimetric method by using platinum capsules (USEPA, 2003).

2.3. Cultivation and counting of bacteria

2.3.1. Strain and culture media

Non-pathogenic *Escherichia coli* (ATCC 35218) strain was used in this study. Different media were prepared for the cultivation and CFU counting: Luria Bertani Broth (LB, Sigma Aldrich, USA) for bacterial growth of *E. coli* in broth at an exponential and stationary growth phase. The recovery of *E. coli* was performed in Tryptone soya agar (TSA, Oxoid, Thermo Fisher Scientific Inc., USA) and/or in Brilliance *E. coli*/coliform agar (Oxoid, Thermo Fisher Scientific Inc., USA). Even if we focused on *E. coli* as an established model (Lin et al., 2014), other strains were considered, including: *Staphylococccus aureus; S. epidermidis*; *Pseudomonas aeruginosa, Enterococcus faecalis*, applying the same materials and protocols for culture.

2.3.2. Preparation of bacterial suspensions

For each experiment, a bacterial suspension was prepared from frozen aliquots of a same stock of *E. coli*. Aliquots were rehydrated into fresh LB medium (5% v/v for *E. coli*) and incubated o/n at 37 °C without shaking, until the bacteria reached the stationary growth phase. After incubation, the absorbance of the suspension was measured at 600 nm to determine the bacterial concentration according to calibration curves obtained previously (DS-11 Series Spectrophotometer-Fluorometer, DeNovix Inc., USA). Therefore, bacterial cells from the same master culture were diluted in the spring waters to obtain the final bacterial suspension for performing the experiments ($1.0 \pm 0.2 \times 10^9$ CFU/ml). To test the resistance capability of human origin bacteria in different thermal spring waters, 50 ml of each thermal water were inoculated with approximately 200 cells of *E. coli*. To test the additional photocatalytic effect on W_3 and BLM waters a final concentration of 10^5 cells/ml was used.

2.4. Bacteriostatic ability test

Water samples were contaminated (10^5 cells/ml) and incubated for 24 h at 37 °C without shaking, in sterile glass bottles, hermetically closed, as previously described (Giampaoli et al., 2012). Just after the inoculum (t_0) and at the end of the 24 h (t_{24}) incubation, each sample was filtered on nitrocellulose membrane (pore size 0.45 µm) (Whatman-GE Healthcare, USA) and transferred aseptically on agar plate, containing TSA (Tryptone soya agar, Oxoid). Each aliquot was spread onto specific agar to allow the bacterial colonies to be counted, by conventional methods (CFU). Negative and positive controls were run in parallel for each experiment. All TSA plates were incubated at 37 °C after 24 h. All experiments were performed at least in duplicate and the concentration of bacteria in the sample were calculated as the average of the number of colonies divided by the volumes inoculated on the specific agar. Based on the dilution factors and intra-laboratory test, the quantification limit was > 25 CFU/ml.

2.5. Photocatalytic test (NP-TiO_2)

2.5.1. Materials

A commercial water-based anatase suspension (ReAir, Varese, Italy) with a TiO_2 nanoparticles (NP-TiO2) content of 0.7–0.9% was used in this study. The bacteria strain used for this experiment was *E. coli*, as previously reported (Kumar et al., 2011; Carrè et al., 2014; Lin et al., 2014). Exposure were performed using LED-based display systems, encompassing an UV source and white light source ranges in the visible spectrum, and in particular a dedicated exposure system including 5 additional LEDs that can cover different ranges of the visible spectrum, 395-400 nm, 450-455 nm, 515–525 nm, 590-595 nm and 620-630 nm (Geckobiotech, Italy).

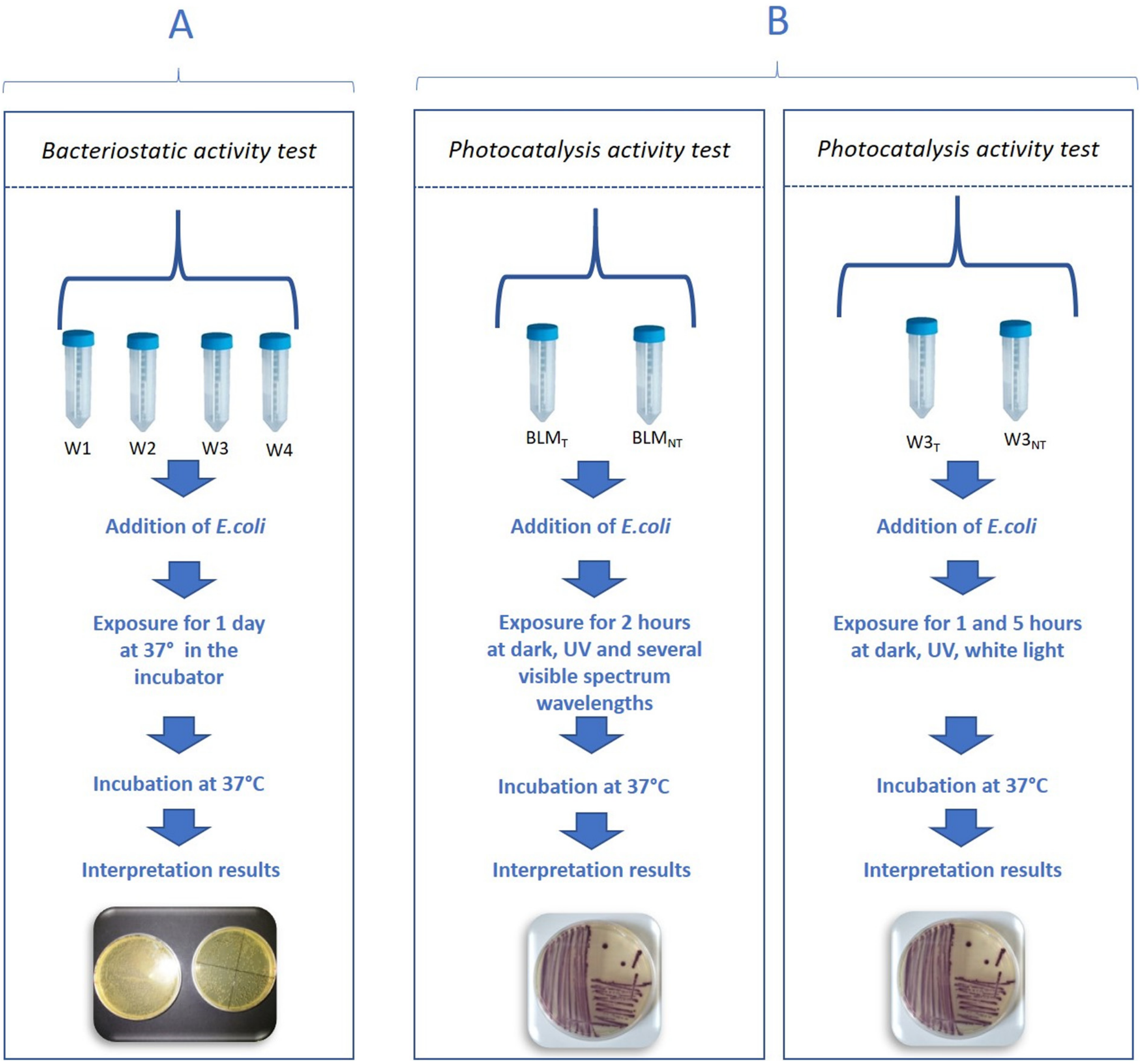


Fig. 1. Summary of study design: (A) A bacteriostatic activity test was performed on several natural thermal waters with different abiotic components ($W_{1–4}$); (B) evaluation of antimicrobial activity after TiO_2 treatment ($_T$) of a low mineral water (BLM) and a high mineral content thermal water (W_3). Each experiment was performed with and without TiO_2 treatment ($_{NT}$) at least in duplicate.

Table 1
Chemical and physical characteristics of the spring waters utilized in the present work. W_1: sulphate water containing sodium chloride and bromine; W_2: slightly sulphate water; W_3: sulphate and bicarbonate water containing alkaline hearth metals; W_4: sulphate-bicarbonate-calcium thermal water; BLM: a drinkable water with low mineral content. NA: not applicable.

Physical/chemical parameter	W1	W2	W3	W4	BLM
Temperature (°C)	69° C	25	54	18	18
Conductibility (μS/cm)	28,291	3640	2590	2730	25.4
pH	6.66	6.7	6.8	6.04	6.9
Fixed residue at 180 °C (mg/L)	20,100	2730	3000	1968	22
CO_2 free (mg/L)	1079	740	871	1710	2
H_2S (mg/L)	< 0.05	2	6	14	–
Potassium ions (mg/L)	531	NA	42	17.6	0.2
Magnesium ions (mg/L)	227	187	125	82	0.42
Sodium ions (mg/L)	6880	40	35	130	1,5
Bicarbonates (mg/L)	1284	1002	840	1920	10
Sulphates (mg/L)	918	180	1181	130	3.2
Bromine (mg/L)	29	< 0.1	< 0.1	< 0.1	–
Arsenic (mg/L)	0.59	NA	0.234	NA	–
Silica (mg/L)	89.4	NA	62	16.5	8.5
Calcium (mg/L)	353	NA	581	483	2.9

2.5.2. Exposure protocol and antimicrobial photocatalytic test

From a same master cell suspension consisting of 10^5 cells/ml of *E coli* with and without TiO_2, 14 ml were transferred into two 60 mm Petri dish plates (7 ml for each one), and independently exposed at room temperature at different wavelength, excluding the lid during exposure. Simultaneously, control samples from the same master culture were incubated for each wavelength in parallel under identical condition but in absence of TiO_2. Furthermore, 2 samples with TiO_2 and 2 without, were incubated in the dark for the same time as the treatment groups (120 min). After each dose of light had been delivered 50 μL aliquots were withdrawn and streaked on TSA agar plates and CFU counted after overnight incubation at 37 °C (Fig. 1).

2.6. Statistical analysis

Each treatment including the blank control was conducted at least in duplicate and the results were presented as mean ± SD (standard deviation). For each sample, data were normalized respect to the reference control without TiO_2. Student's *t*-test was used for pairwise comparisons. Statistical analyses were performed using the SPSS 22.0

(SPSS for Windows; SPSS Inc., IBM, Chicago, IL, USA) at a significance level < 0.05.

3. Results and discussion

Several strategies for managing hygiene in natural SPA pools were described by different authors, highlighting potentials and limits for a sustainable management (Valeriani et al., 2018a). Alternative approaches comprise nanotechnologies, photocatalytic systems, advanced filtration and other physical methods. Different filtration strategies showed effectiveness but impose an accurate maintenance and may need prefiltration steps due to the presence of aggregates (Gitis and Hankins, 2018). Innovative and promising materials are continuously developed to reduce biofilm formation in pool surfaces and along the pipeline plant (Valeriani et al., 2017a). The specific interaction between the oligotrophic ecosystems and the physicochemical properties plays an essential role on the survival of contaminating waterborne bacteria -including pathogens- in these extreme environments (Sevillano et al., 2018). Some studies reported a direct relationship between mineralization levels or H_2S concentration, and the survival of different bacterial species in natural SPA waters (Giampaoli et al., 2013; Serrano et al., 2012). Starting from these considerations, the native antimicrobial proprieties of independent natural SPA waters were investigated, further confirming the presence of an intrinsic disinfectant activity, probably due to salt concentration or to the presence of specific elements such as Sulphur, Arsenic, Iron, or pH. In order to asses a possible additional increment of the native bactericidal effect, a nanotechnological approach, based on TiO_2 photocatalysis (NP-TiO_2), was applied.

As shown in Fig. 2, the survival of *E. coli* after 24 h of incubation in natural SPA waters, revealed a 30–80% reduction, depending on the native chemical composition of the different waters; in particular: i) 80% in W_3; ii) 75% W_1 and W_4; iii) 30% in W_2. The presented data show a general reduction of viable and cultivable bacterial cells in all thermal spring water samples respect to a low mineral content spring water (BLM), or to isotonic (SS) and buffered (PBS) solutions, respectively used as controls. W_2 water sample showed the lowest death rate. This result agrees with other studies, suggesting a major role for Sulfidic acid or salt concentrations, that are more elevated in W_1, W_3, W_4 respect to the W_2 water composition, as reported in Table 1 (Serrano et al., 2012; Lo Nostro et al., 2005; Giampaoli et al., 2012).

Abiotic factors, such as temperature, pH, conductibility or mineral content, have a relevant influence on the natural microflora present in the ecosystem (Valeriani et al., 2018b). Indeed, an equilibrium is reached so that both the living (biotic) and the non-living (abiotic) intrinsic components characterize that specific SPA water, impacting on the survival of allochthonous bacteria (Mathur et al., 2007; Sevillano et al., 2018). Observed results, suggest that some natural SPA waters may already have an intrinsic resistance to polluting bacteria proliferation, opening up new scenarios for the application of alternative, less invasive water-treatment strategies, than traditional chemical disinfection. In this context, we asked if the application of innovative and sustainable approaches could somehow increase this intrinsic antimicrobial property.

In order to test alternative solutions to control bacterial contamination, we selected some photocatalytic materials, and performed test using TiO_2. We already showed a higher resistance to waterborne biofilm in materials covered with TiO_2 and used for inner coating of pools or its pipeline (Valeriani et al., 2017a). Here, we evaluated the incremental antibacterial effect by direct addition of a NP-TiO_2 solution. We selected the natural SPA water that showed the highest antibacterial activity (W_3) and a drinkable spring water with a low mineral content (BLM), confirming an incremental antibacterial effect, as shown in Figs. 3 and 4.

Interestingly, the antimicrobial effect in W_3, already elevated (80%) without TiO_2, further increased with UV light and NP-TiO_2 (99%), as showed in Fig. 3. In particular, after 5 h of exposure, the untreated samples in the dark, exposed to UV, and exposed to white light source ranges in the visible spectrum show a reduction of 81%, 79% and 89% respectively. With the addition of the TiO_2 treatment, we see a reduction of 90%, 99% and 84% respectively. Specifically, an increase in bacterial reduction in the presence of TiO_2 was, also, found at 1 h of incubation (35%, 34% and 54% in untreated respect to 66%, 79% and 72% in treated samples).

A CFU reduction was observed, also, under dark conditions both at 1 h and 5 h of exposition. Indeed, the dark cannot be absolute, since the pipetting and handling have to be done in the presence of light, as all the preparation phases of the experiment, including the collection of the aliquots. Moreover, this unexpected effect was previously described also by other authors, suggesting that NP-TiO_2 particles exhibit a bactericidal activity even in the dark; this mechanism is supposed to be influenced by the interaction between NPs and bacteria, with consequently microbial death for interruption of the nutrient uptake or waste disposal processes (Tahir et al., 2016; Erdem et al., 2015). It is well known that UV irradiation of TiO_2 induces production of Reactive Oxygen Species (ROS) and different studies have identified that the action of TiO_2-based photocatalysis both under illumination or dark, can occur on different cellular key-components, such as lipids and proteins (Carré et al., 2014; Lin et al., 2014). Under dark conditions, the antibacterial photocatalytic activity can induce lipid peroxidation by ROS, acting on membrane fluidity and compromising cell integrity (Erdem et al., 2015). The same TiO_2-driven bactericidal effect was observed also with other microorganisms (data not shown) and also on low mineral content waters, with a percentage of reduction over 92% ($p < 0.05$) after 2 h exposures (data not shown). The whole of the observed data suggests that NP-TiO_2 have an intrinsic antibacterial activity that is not evident only under UV exposure. Indeed, the application of the titanium dioxide as photocatalytic compound was initially restricted to UV, also due to the low quantum yield (Tabaei et al., 2012). However, several studies have focused the attention on the

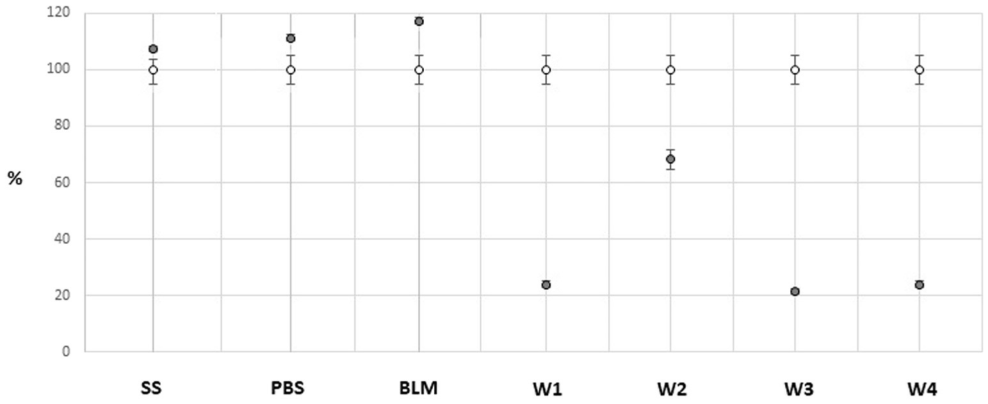


Fig. 2. Bacteriostatic activity in natural SPA waters with different abiotic components and a spring drinkable water with low mineral content (BLM): *E. coli* survival after 24 h of incubation at 37 °C. As additional controls were used 0.9% Saline Solution (SS) and Phosphate Buffer Saline (PBS). Values of CFUs are normalized on the t_0 value and data are expressed as percentage of survival. Mean value and standard deviation of experiments in triplicate.

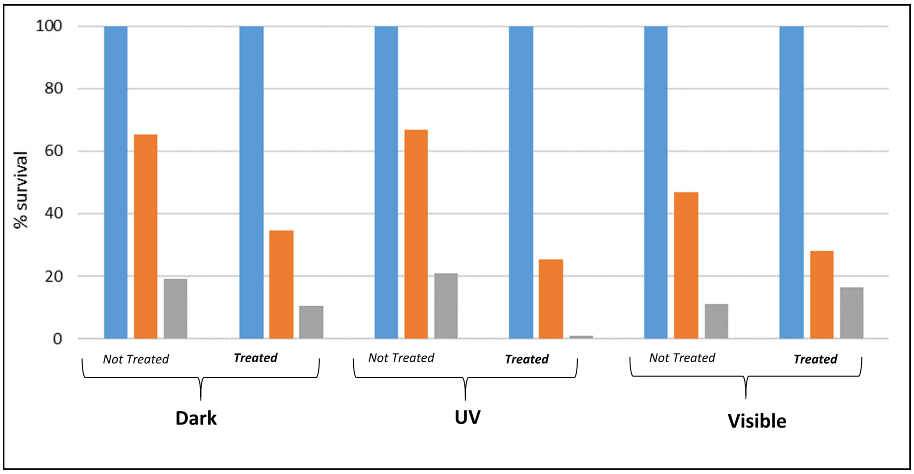


Fig. 3. Increment of the native antibacterial activity by TiO_2 treatment. Experimental test with high mineral content water (W3, sulphate bicarbonate water containing alkaline hearth metals) after contamination with *E. coli* and treatment with TiO_2 nanoparticles. Data were expressed as survival percentages of *E. coli* bacteria after exposition to dark, ultraviolet (UV) and white colour light at visible wavelengths. Data collected at different times of exposition: blue bars before TiO_2 exposition, orange bars at 1 h and grey bars after 5 h of TiO_2 exposition. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

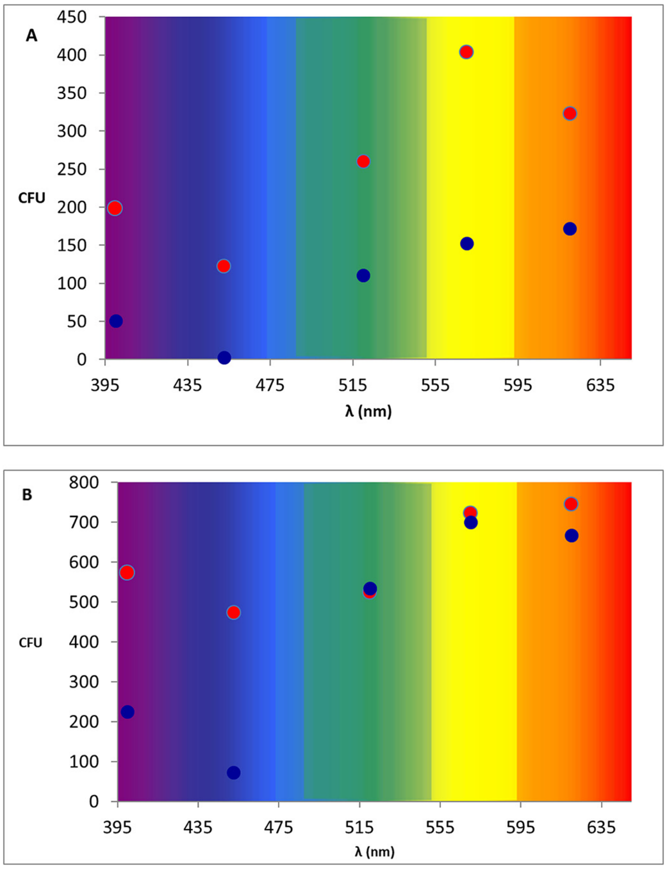


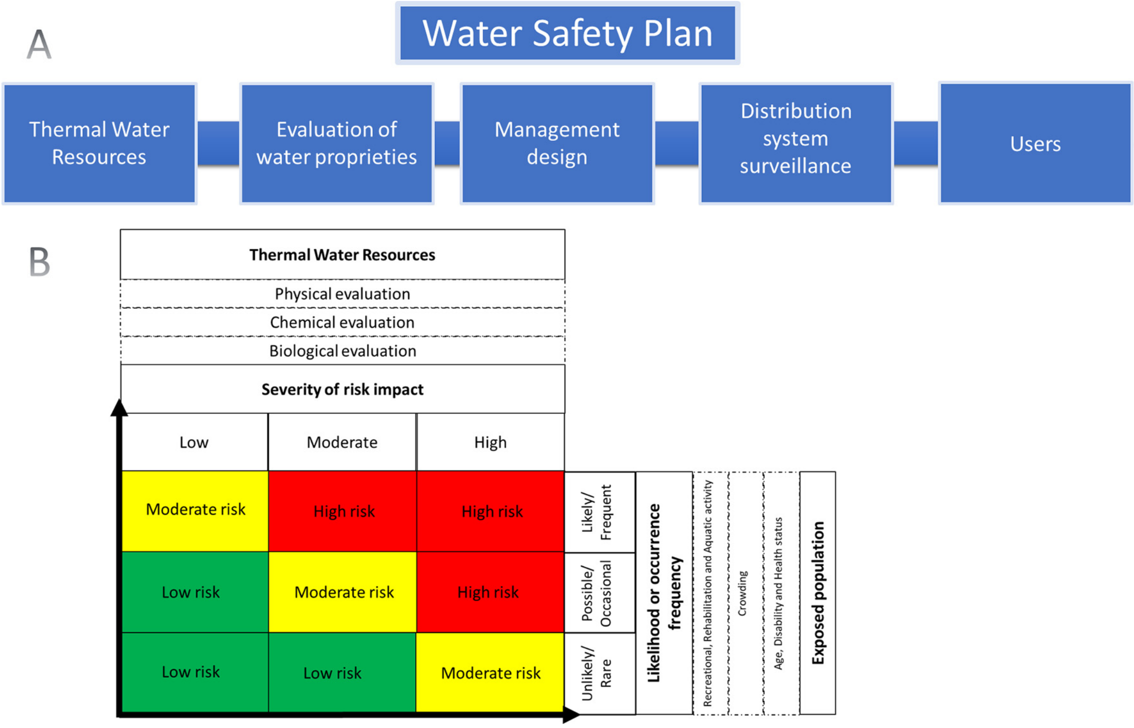
Fig. 4. Experimental test of low mineral content water contaminated with *E. coli* and treatment with (blue circle) or without TiO_2 nanoparticles (red circle). Data were expressed as means of CFU x 10^2 count on agar plates incubation after 120 min exposure (T_{120}) at several wavelengths of the visible spectrum. Starting *E. coli* concentration at T_0: $< 10^5$ (A) and $> 10^5$ (B). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

possibility of activating titanium dioxide even under illumination with visible spectrum wavelengths, with cost reduction and allowance of its use also in the presence of bathers. Several strategies have been adopted to promote the separation efficiency of electron pairs and to shift the absorption average of TiO_2 in the visible spectrum (Nasr et al., 2018; Higashimoto, 2019; Sethi et al., 2014; Carré et al., 2014). Therefore, we investigated the bactericidal effect under different wavelengths. The action of NP-TiO_2 was evaluated using the same approach, but at different wavelengths within the visible spectrum, ranging 395–630 nm. An over 98% decrease in the number of CFUs was observed for samples exposed to blue-light wavelength between 450 and 455 nm (Fig. 4a). The blue-light bactericidal effect was present also with a higher contamination load (*E. coli* $> 10^5$ CFU/ml), even if at a lower level (85%); moreover, when simulating higher contaminations, the NP-TiO_2 effect was present only at UV and blue-light wavelengths, being lost for the other colours of the visible spectrum (Fig. 4b). The whole of these data supports the effectiveness of exposure to sun-light UV-Blue components ($<$ 470 nm) but poses several concerns on the success of this approach in indoor environments with artificial warm light. Indeed, the energy required to excite an electron from the valence band to the TiO_2 conduction band is 3.35 eV, which is equivalent to the energy of a photon with a wavelength of about 360 nm. For this reason, the bactericidal effect of Titanium dioxide decreases with wavelengths exceeding 450 nm due to the lower energy and therefore lower generation of free radicals. If artificial UV light cannot be used in presence of bathers due to the mutagenic effect, the blue-light may represent a safe, effective and promising alternative to activate NP-TiO_2 in natural SPA pools. Moreover, a wavelength range between 450 and 455 nm can exhibit a direct antimicrobial effect, as previously reported in recent literature and slightly detected also in our experiments (Wu et al., 2016; Wang et al., 2017; Gwynne and Gallagher, 2018; Dai, 2017).

The whole of experimental data and literature reports strongly supports the potential role of photocatalytic materials in enhancing the intrinsic antibacterial activity of natural SPA waters. Further studies are needed to optimize and validate these promising strategies, testing the influence of other parameters such as water flow rate, turbidity, temperature, oxygen content, organic compounds concentration, presence of different salts or chemical elements. Other limits of the present study are related to the selection of the photocatalysis-based approach, without considering the potentials of a synergic combination of different other techniques, such as ultra-filtration, microwave or ultrasound-based strategies (Valeriani et al., 2018a; Zou et al., 2017).

However, the proposed general model can support a personalization of the WSP by the integration of any suitable candidate technique, requiring its validation on field and potential inclusion in future guidelines or regulations. The establishment of an appropriate treatment for the recreational use of natural spa waters in pools will not only protect users and the spring water composition by avoiding chemical addition or disinfection by-products generation, but it will also sustainably impact on environment, by allowing recirculation and reducing the depletion of the aquifer (WWAP, 2018).

The design of a dedicated Water Safety Plan in these facilities, should, thus, provide a personalized process flow, starting from the analysis of water resources (Fig. 5a). Indeed, each water may highly differ from another and the traditional guidelines for treating



Low	Moderate	High	
Moderate risk	High risk	High risk	Likely/ Frequent
Low risk	Moderate risk	High risk	Possible/ Occasional
Low risk	Low risk	Moderate risk	Unlikely/ Rare

Fig. 5. A proactive risk-management strategy to be performed for facilities that use thermal water resources for recreational uses. (A). The proposed Water Safety Plan approach should start from the evaluation of the specific natural properties of the SPA water: i) identify protective factors and risks factors related to the water supply system; ii) define a dedicated plan to manage water hygiene and its surveillance and maintenance by considering supply/dilution factor and alternative treatments. (B) Starting from water properties, implemented treatments and planned monitoring, the classification of risks should focus on the target population and the intended use of the pool allowing: identification of risk priorities to the final aim of minimizing risks, through an adequate management.

swimming pools may often not be applicable or be ineffective or even dangerous (WHO, 2006, 2019). Moreover, unlike traditional pools fed with fresh water, where recirculation plays a predominant role (about 70–80%), natural spa pools may benefit of a higher dilution factors related to the availability of natural sources that are often copious and abundantly flowing. The higher water renewal -and therefore the higher dilution of pollutants- is an additional element that brings the recreational use of spa waters much closer to coastal waters hygiene than to the management of standard swimming pools facilities (Giampaoli et al., 2012; WHO, 2003). A personalization of the WSP is necessary and should consider several factors, including the specific water composition and availability, the distribution system (e.g. engineering and pipeline materials), the intended use (e.g. ludic-recreational, rehabilitative, medicinal, adapted physical activity, wellness, etc.), the potential sources of contamination (both anthropic and environmental), the exposed population (age, disability, immunodepression, health status), and the allowable crowding thresholds. A schematic summary of this general approach is described in Fig. 5b and it must include a surveillance plan by advanced monitoring after hazard analysis of critical control points (WHO, 1999, 2019). Moreover, independently of the availability of suitable technologies for water hygiene, this approach requires awareness of the management and education of users, to avoid and minimize risks.

In conclusion, hygiene management of natural SPA pools needs a sustainable approach, based on alternative strategies. Photocatalytic nanotechnologies may represent a promising solution to enhance the native antimicrobial properties of SPA waters and support development of sustainable water safety plans.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

Not applicable.

Declaration of competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Authors' contributions: FV and VRS conceived, designed and developed the study. FV, GG and LMM performed the experiments and the statistical analyses of data. VRS and FV wrote the paper. All authors edited and approved the final manuscript.

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