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### Hydrogen sulfide in thermal spring waters and its action on bacteria of human origin

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#### ABSTRACT

Hydrogen sulfide ( $H_2S$ ) is a molecule dissolved in many thermal spring waters at variable concentration. The  $H_2S$  effects of thermal waters treatments have long been studied, for dermatological and clinical treatments, but its role in recreational waters was never investigated. The use of sulfur spring waters in pools raises concerns related to disinfection by oxidants. The aim of this work is to evaluate the survival rate of microbial species in waters with different titers of  $H_2S$ . Four selected thermal waters collected from Italian springs, belonging to different chemical categories, have been tested in comparison to Tyrrhenian sea water and natural mineral bottled water. Results show inhibition properties on bacterial proliferation that seem related to  $H_2S$  concentrations. To further asses this phenomenon  $H_2S$  was added to thermal and natural mineral waters. The results strongly support a bactericidal role of  $H_2S$  in thermal spring waters used for recreational purposes. These observations open up new perspectives for a disinfectant role of  $H_2S$  in pool treatment and management.

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### 1. Introduction

Hydrogen sulfide (H<sub>2</sub>S) is a colorless, flammable gas with a "rotten egg" smell. It is one of the principal components in the natural sulfur cycle. Bacteria and fungi release H<sub>2</sub>S during the decomposition of sulfur-containing proteins or by the direct reduction of sulfate  $(SO_4^{2-})$ . H<sub>2</sub>S is also consumed by bacteria found in soil and water by the oxidation of hydrogen sulfide to elemental sulfur. Photosynthetic bacteria can oxidize H<sub>2</sub>S to sulfur and sulfate in the presence of light and the absence of oxygen [1,2]. H<sub>2</sub>S is commonly emitted from volcanoes, stagnant or polluted waters, hot springs, and underwater thermal vents. These natural sources account for about 90% of the total hydrogen sulfide in the atmosphere. Although H<sub>2</sub>S has been studied for its toxic properties, several recent experimental evidences suggest that H<sub>2</sub>S may play a prominent role in normal physiology and pathophysiology. Therefore, many therapeutic targets exist for H<sub>2</sub>S therapy, including cancer, heart failure, organ transplant, peripheral artery disease, inflammatory bowel disease, Alzheimer's disease, acute myocardial infarction (MI), stroke, atherosclerosis, hypertension, erectile dysfunction, metabolic syndrome, diabetes, and thrombosis [3-8].

 $H_2S$  is dissolved in many thermal spring waters at variable concentration, used by centuries in different countries [9]. In general, spring waters have different chemical compositions, which can vary

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considerably depending on the location of the source (e.g. near the sea, volcanic, geothermic or mountain areas, etc.). The thermal waters are described as salty, sulfurous, bicarbonate, sulfated, carbonic, arsenical, or ferruginous, on the basis of their mineral composition [10]. The chemical concentration of thermal water is directly correlated to its therapeutic effects. To date, the chemical elements and mechanisms involved in the health benefits of hot springs remain still unclear [11].

Several authors reported bactericidal and antifungal activity from thermal spring waters with special focus on dermatological diseases [11–14]. Large importance has been dedicated to the pH level or to the presence of specific cations or anions in the water. The H<sub>2</sub>S may be present under certain conditions as different reactive sulfur species. For example, the interaction between sulfur and oxygen radicals leads to the formation of subproducts, such as pentathionic acid  $(H_2S_5O_6)$ , which may represent the source of the antibacterial and antifungal activity of thermal water on the skin [12-15]. This specific bactericidal activity can be particularly important in the management of thermal waters for recreational use [16]. Pools used for thermal baths in "salus per aquam" resorts (SPA) may be characterized by bacteria contamination due to overcrowding pool conditions. The microbiological risk is commonly countered by disinfection procedures. Chlorination or ozone treatments, largely adopted in conventional swimming pools, are not applicable to SPA pools or swimming ponds [17], due to the high level of reducing agents and salts present in natural thermal waters. Thus oxidative methods should not be applied in thermal waters as they may alter chemical components and,

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