SPORULATED SULPHITE-REDUCING ANAEROBES IN NATURAL MINERAL WATER QUALITY ASSESSMENT



Daniela Ferreira, Maria João Ribeiro, Maria Sameiro Maciel, Carla Coelho and Cristina Pizarro

Laboratório de Microbiologia, Departamento de Saúde Ambiental, Instituto Nacional de Saúde Doutor Ricardo Jorge, Rua Alexandre Herculano 321, 4000-055 Porto, Portugal

Natural mineral waters have exceptional characteristics and known since ancient times to benefit human health in several disorders (Lourenço, 2012). According to Portuguese legislation (Decree-Law 156/98), these waters are defined as bacteriologically safe, with stable physico-chemical characteristics at the source, within the natural variation range, and which differentiate from usual drinking water given their possible beneficial health effects. Currently the use of Portuguese natural mineral water for bottling and thermal treatments is widespread. Therefore, bacteriological control is mandatory by legislation (Decree-Law 156/98; Ordinance 1220/2000) and done regularly in order to guarantee water quality. All waters covered by these legislations must be bacteriological wholesome, defined by indicator organisms. The quality requirements include the enumeration of sporulated sulphite-reducing anaerobes, which should be absent from these types of waters.

The aim of the present study is to evaluate the importance of sporulated sulphite-reducing anaerobes on mineral water for bottling and thermal use, according to the laboratory results obtained during nine years.

Methodology

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Statistical study conducted from a total 5643 water samples for bottling (natural mineral water: 1154; spring water: 2475) and thermal use (for mucosae contact: 1087; baths: 927), located in the Galiza-Trás-os-Montes and Centro-Iberian geotectonic zones, analyzed during the years 2007 to 2015.

Bacteriological quality indica-

tors: sulphite-reducing anaerobe (SRA) spores, heterotrophic plate counts (HPC) at 37 and 22°C, coliform bacteria, *Escherichia coli*, enterococci and *Pseudomonas aeruginosa*.

In this mineral water study, the data from spring water for bottling was analyzed separately.

Results **Natural Mineral Water Spring Water** \prod III ,1,3% 61,8% 41,1% 3,6% 71,5% 28,5% 40,1% _3,6% 0,2%_ ■HPC at 22°C ■SRA spores **■**Enterococci ■E. coli ■ Coliform bacteria ■P. aeruginosa ■ Conform ■ Non-conform ■HPC at 37°C Other indicators than HPC

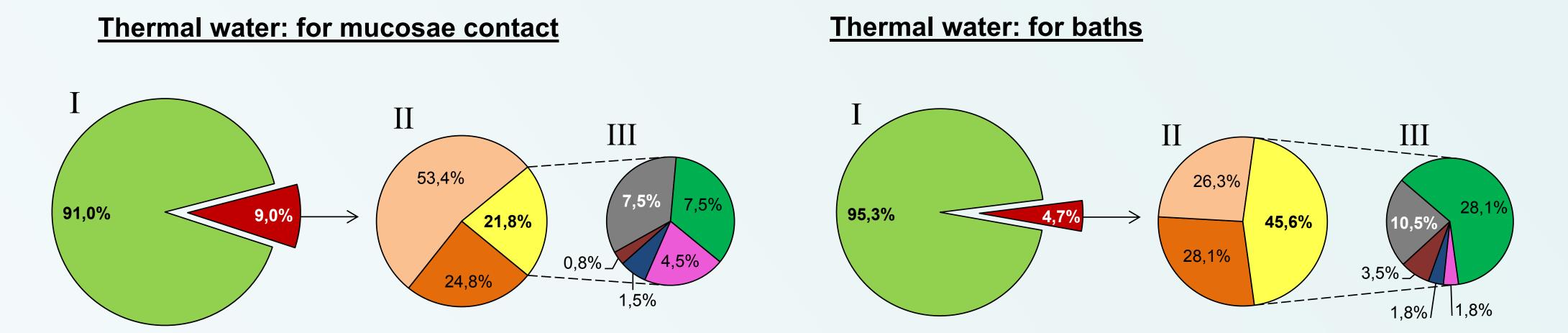


Figure 1. Percentage of total sample conformity (pie I) and frequency of the bacteriological indicators (pies II and III) detected in each type of water. Legend applies to all charts. SRA: sulphite-reducing anaerobes; HPC: heterotrophic plate counts.

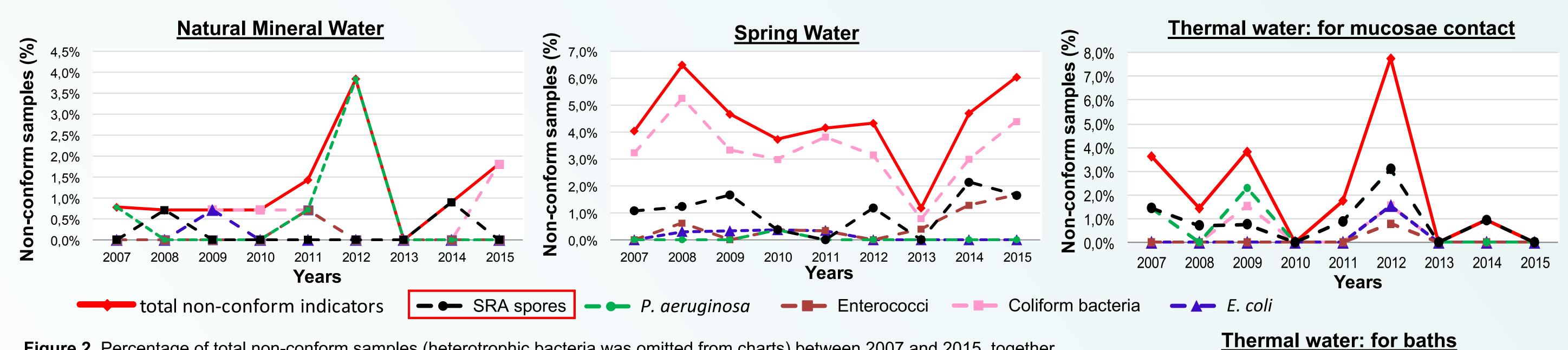
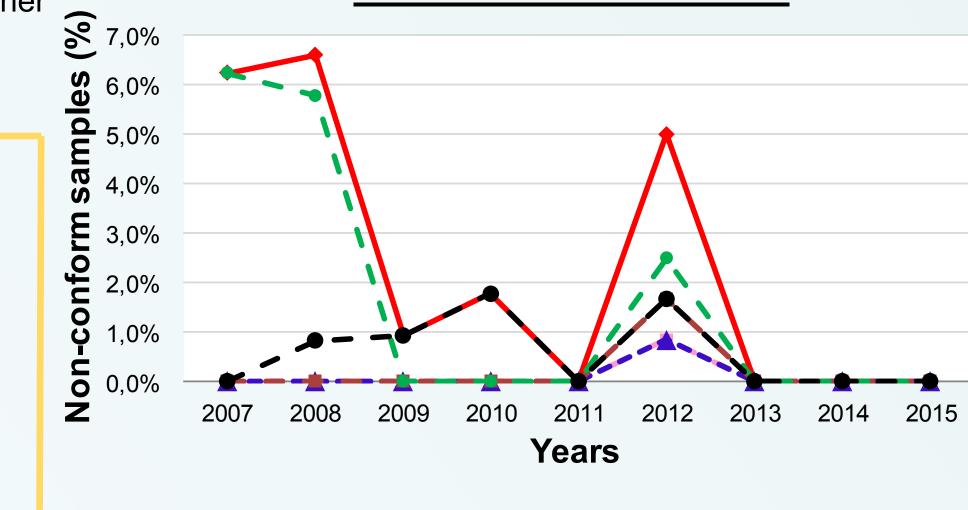


Figure 2. Percentage of total non-conform samples (heterotrophic bacteria was omitted from charts) between 2007 and 2015, together with the parcel corresponding to each bacteriological indicator. Legend applies to all charts. SRA: sulphite-reducing anaerobes.

Table 1. Number of non-conform samples with or without SRA spores.

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	Natural Mineral Water	Spring Water	TW for mucosae contact	TW for baths	Total waters
SRA spores only	2	15	9	5	31
SRA spores with other indicators	0	10	0	1	11
Other indicators without SRA spores	327	228	89	38	682
TOTAL	329	253	98	44	724

SRA: sulphite-reducing anaerobes; TW: thermal water.



Conclusions

The water samples analyzed have a low contamination occurrence, no more than 5% when accounting for all indicators, excluding heterotrophic bacteria.

The level of mineral water contamination was variable over the years. Besides heterotrophic bacteria, *P. aeruginosa* largely contributed to the sample non-conformity (except in spring water), SRA spores emerged in second or third positions.

Overall, less than 1% of total non-conform water samples correspond to the presence of sporulated sulphite-reducing anaerobes.

Most mineral water samples contaminated by SRA spores were absent from the other indicator microorganisms.

The frequency of SRA spores in our water sampling was low. Given the extent of national use of these waters, SRA spore assessment may still be useful to accomplish quality assurance because they are a long-lived indicator.