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# Influence of water temperature on the growth of *Legionella* in real environmental samples: Surveillance in South Tyrol 2011-2016

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## Introduction

The surveillance of water samples for monitoring the presence of *Legionella* in sensible structures is critical in managing *Legionella* risk, as foreseen in the Italian guidelines published in 2015. The Biological Laboratory of APPA Bolzano is the reference *Legionella* laboratory for South Tyrol: here are analysed all water samples collected in the province. The Biological Laboratory has therefore a privileged point of view on the presence and distribution of *Legionella* in the environment (hot waters, plants, …) and has collected in the years a huge database.
We present here the results of data collected from real water environmental samples in the last 6 years (2011-2016) in ca. 400 structures spread in South Tyrol with focus on the temperature of water samples.

## Methods

Water samples conferred to the Laboratory were either official probes sampled by the control authorty during the ordinary and/or extraordinary monitoring activities or water samples from self-monitoring plans of hotels, retirement homes, hospitals, private citizens. All samples were analyzed by accredited ISO 11731. Most of them were accompanied by supplementary information about kind, mode and date of last plant treatment, water temperature, notification of a case of illness.

## Results

A total of 5784 samples were analyzed along 6 years. 61% of the samples were *Legionella*-free and 10% contained 50-100 cfu/L, which are values below the attention threshold indicated in the Italian guidelines. In the remaining 29% of samples *Legionella* was found in amounts >100 cfu/L (Fig. 1).
The same ratio between not contaminated/contaminated samples was found in the 3826 samples accompanied by additional information on water temperature. We focused out attention on samples containing >100 cfu/L and grouped the data of these "positive" samples in three ranges of water temperature (Fig. 2A, 2B).
Temperatures of most of the samples containing *Legionella* were in the range 20-50 °C, which is the optimum of temperature for *Legionella*'s growth (as indicated in Italian Guidelines 2015). Nevertheless, a high number of samples (336) collected with temperatures higher than 50 °C were positive for the presence of *Legionella* (Fig. 2A). Grouping the data considering a different range of temperature as optimal for *Legionella* survival and growth (25 -55 °C), the number of contaminated samples increased up to 980, while only 68 were the samples containing *Legionella* collected at temperature higher than 55 °C (Fig. 2B). In fig. 3 are reported the number of samples belonging to each of the five temperature ranges: filled histograms represents the samples with counts >100 cfu/L, pointed histograms those with counts ≤100 cfu/L. The microbial load (grouped in three classes) of the positive samples is reported in Fig. 4 as percentages of positive samples. Our results clearly indicate that a considerable number of water samples collected at temperatures between 50 and 55 °C show the presence of *Legionella* in amounts that impose to take measures for managing *Legionella* risk. The recommended water temperature of 50 °C in the plants – as in the guidelines - seems not to be safe enough in avoiding bacterial proliferation.

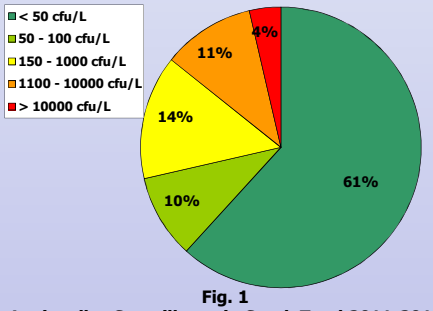


**Fig. 1**
***Legionella* - Surveillance in South Tyrol 2011-2016**
**(5784 water samples)**

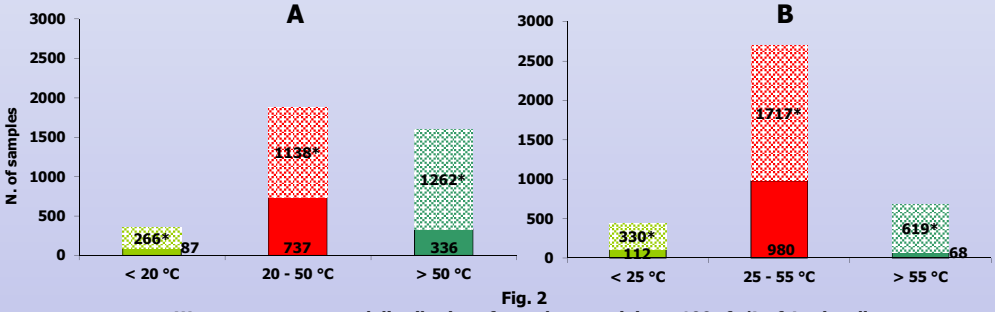


**Fig. 2**
**Water temperature and distribution of samples containing >100 cfu/L of *Legionella***
**(3826 total samples, * N. of samples with values ≤100 cfu/L )**

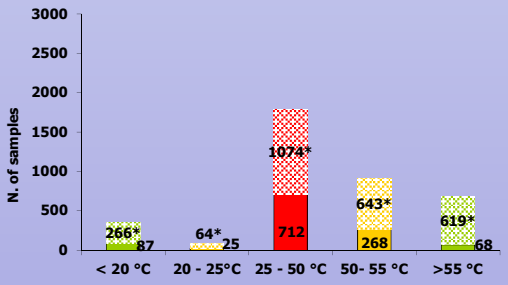


**Fig. 3**
**Water temperature and distribution of 1160 samples containing >100 cfu/L of *Legionella***
**(3826 total samples, * N. of samples with values ≤100 cfu/L )**

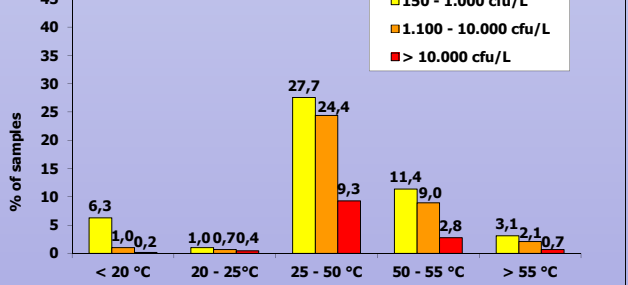


**Fig. 4**
**Water temperature and presence of Legionella**
**(1160 samples containing >100 cfu/L of *Legionella*)**

## Conclusions

The guidelines indicate the temperature range 20-50 °C as ideal for *Legionella* growth, therefore suggesting a water temperature of 50 °C for preventing bacterial development. We have shown that a not negligible amount of *Legionella* can be recovered even at higher temperatures, belonging the 23,2% of total samples contaminated by more than 100 cfu/L of *Legionella* to the group of samples whose temperature was between 50 and 55 °C.
We therefore propose to change the temperatures recommended in the Italian guidelines, changing them according to UNI EN 806-2:2008 that recommend keeping cold water below 25 °C and hot water above 55 °C.