

## Minimum inhibitory and minimum bactericidal concentrations of boron compounds against several bacterial strains

Murat Tolga YILMAZ

**Aim:** Boron compounds are essential micronutrients for many organisms. However, they negatively affect plant, soil, and water microflora if excessive amounts exist in irrigation water. Therefore, this study aimed to define the minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of boric acid and borax by selecting the bacteria that can survive in all environments.

**Materials and methods:** The antibacterial efficacy of boric acid and borax against several bacterial strains was evaluated 3 times with the macrodilution broth method.

**Results:** The MICs and MBCs of boric acid were obtained as 3.80 mg/mL, 3.80 mg/mL, 7.60 mg/mL, and 7.60 mg/mL against the bacterial activities of *Staphylococcus aureus*, *Acinetobacter septicus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, respectively. The MICs and the MBCs of borax were obtained as 23.80 mg/mL, 23.80 mg/mL, 47.60 mg/mL, and 47.60 mg/mL against the above bacteria, respectively.

**Conclusion:** Current results suggest that water containing boron compounds at levels lower than the MICs and MBCs can be used for irrigation. It also seems feasible to use this approach to decide whether irrigation water is needed to remove excessive boron compounds. Furthermore, current MIC and MBC results shed light on the medicinal use of boron. Future studies may open a new avenue in the use of boron compounds as novel antibiotic agents.

**Key words:** Boron, biological treatment, MIC and MBC, bacteria

### Introduction

Boron is a minor element, which is used in many different areas such as fertilizers, insecticides, corrosion inhibitors, buffers in pharmaceutical compounds and dye stuff production, and nuclear reactors where anthropogenic water-soluble boron compounds are discharged into aqueous environments (1,2). Boron compounds are essential micronutrients for many organisms and play important roles in plant life (3). However, in large amounts, boron is also toxic to living cells. The gap between boron deficiency and toxicity is fairly small for all living organisms (4,5). Boron is involved

in quorum sensing, an important mechanism in establishing antimicrobial activity (6,7).

If the amounts of boron in irrigation water are excessive, it negatively affects plant, soil, and water microflora. Because of these negative effects, the removal of excessive boron levels from water is one of the most important and problematic areas in environmental engineering. Conventional biological treatment methods are of limited value because of the toxic effects of boron compounds on microorganisms. Therefore, more complicated and advanced technologies, such as ion exchange methods, are used in boron removal from wastewater

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(8,9). So far, no data have been published to show the minimum boron concentration that does not preclude biological treatment of water. Therefore, the main aim of this study was to find the maximum boron concentration that does not prevent the biological treatment of water.

The bacterial agents used for the biological removal of boron compounds are also common pathogens for human diseases. The idea of implementing boron as an antibiotic agent is not new (10,11). However, boron compounds are generally used as antiprotozoal agents (10). Thus far, there are limited data about the antibacterial effects of boron-containing protein synthesis inhibitors (12). Thus, another aim of this research was to find out the minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of boric acid and borax such that the bacteria could survive in all environments. The effect of pH value on MICs and MBCs was also studied, since the addition of boric acid and borax into a solution changes its pH value.

## Materials and methods

The antibacterial effects of boric acid and borax were analyzed according to the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS) (13). The *Staphylococcus aureus* ATCC 25923, *Acinetobacter septicus* DSM 19415, *Escherichia coli* ATCC 35218, and *Pseudomonas aeruginosa* ATCC 27853 strains were used for the test.

In microbiology, the MIC is the lowest concentration of an antimicrobial that inhibits the visible growth of a microorganism after overnight incubation (14). MICs were defined as the lowest concentration of boric acid and borax inhibiting visible growth of the bacteria. The MICs of boric acid and borax were conventionally determined in triplicate for each strain by the macrodilution broth method as described by the NCCLS (Figure 1a) (13). Serial dilutions of both boric acid and borax were prepared in macrodilution tubes with concentrations ranging between 1/2 and 1/64. Bacterial suspensions were adjusted to the logarithmic-phase growth to match the turbidity of a 0.5 McFarland standard, yielding approximately  $10^8$  CFU/mL. The same

amounts of bacteria were added to all tubes and the tubes were incubated at 37 °C for 24 h. Each tube was examined for growth and compared to the control. Bacterial suspension added to a tube filled with nutrient broth without boric acid and borax was used as a positive growth control. A tube not containing nutrient broth was used as a negative growth control. The absence of growth was defined as antibacterial activity.

Samples not containing boric acid and borax were also prepared to demonstrate the effect of pH. Bacterial inoculum was prepared by suspension of freshly grown bacteria in sterile saline (0.9% NaCl w/v) (14,15) and was adjusted to a 0.5 McFarland standard.

For each test, *Staphylococcus aureus* ATCC 29123 and *Enterococcus faecium* ATCC 6057 were used as control strains in order to compare the activity of boric acid and borax in inhibiting the growth of bacteria. All results for these strains were in the quality control ranges published by the NCCLS.

The MBC is the lowest concentration of antibiotic required to kill a particular bacterium (14,16). The 6 dilutions were run in duplicate for the MBC test. At the end of 24 h of incubation, the tubes were read for the MIC and then the MBC was determined by sampling all the macroscopically clear tubes (1 dilution below the MIC was used for the levels to be assessed in the MBC assay) and the first turbid tube in the series (Figure 1b). Running dilutions above the known MIC permits detection of tolerance to normally bactericidal agents (boric acid and borax) (17). The suspension was inoculated onto plates of blood agar (Merck 1.10886). The plates were filled with 1 M boric acid and 1 M borax to a height of approximately 9 mm. The plates were incubated for 24 h at 37 °C. Each experiment was carried out 3 times and was correlated against the controls.

## Results

The antibacterial activities of boric acid and borax against standard *Staphylococcus aureus* ATCC 25923, *Acinetobacter septicus* DSM 19415, *Escherichia coli* ATCC 35218, and *Pseudomonas aeruginosa* ATCC 27853 strains are presented in Figures 2 and 3.

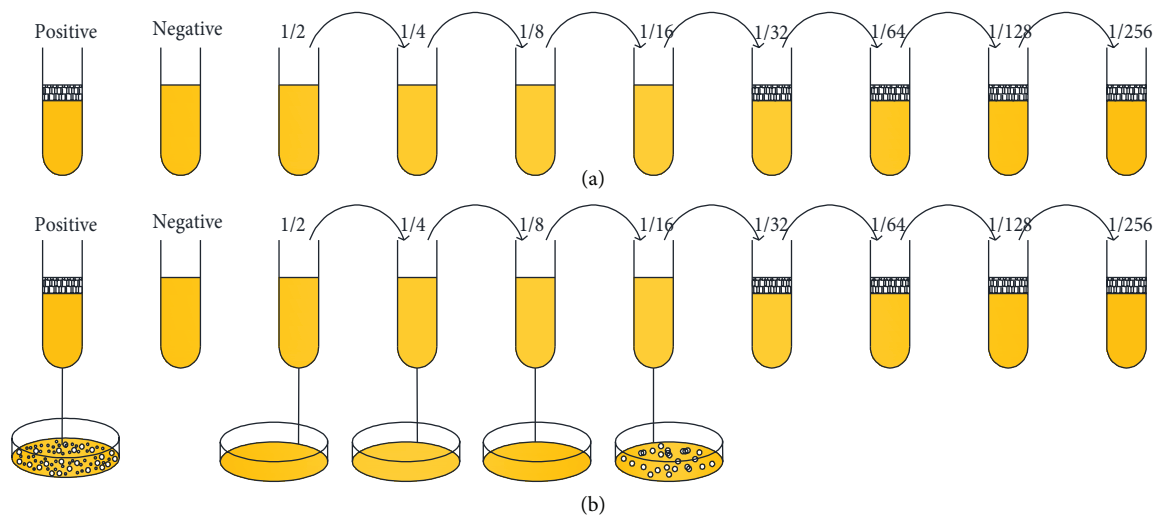


Figure 1. Macrodilution tube method for a) the MIC assay and b) the MBC assay.

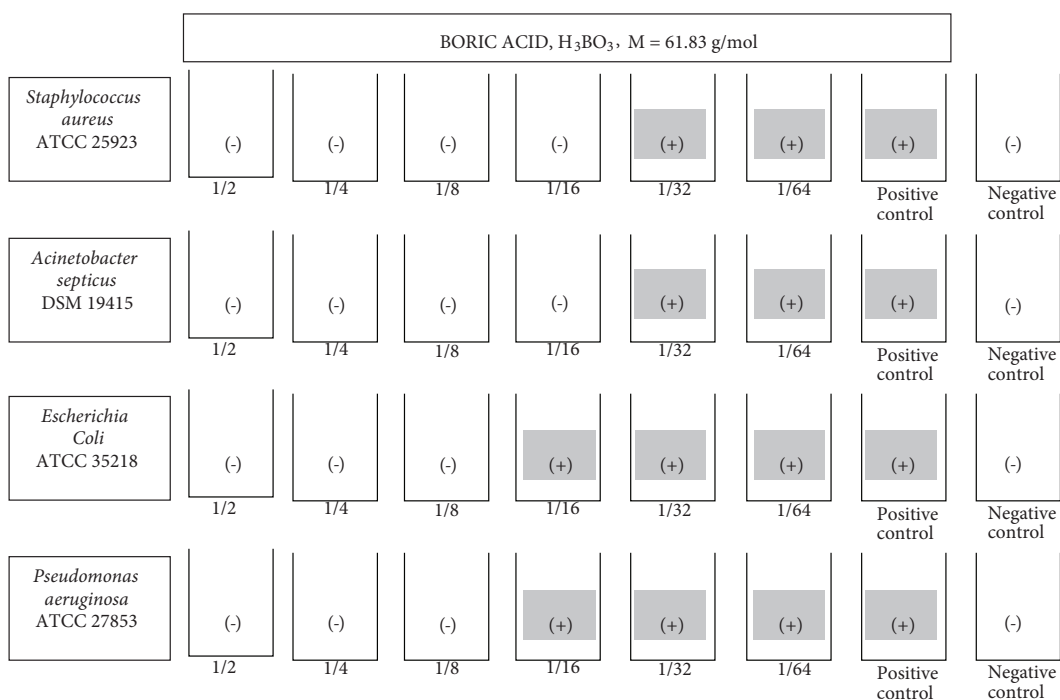


Figure 2. Minimum inhibitory concentrations of boric acid.

The MICs of boric acid obtained for *Staphylococcus aureus* ATCC 25923, *Acinetobacter septicus* DSM 19415, *Escherichia coli* ATCC 35218, and *Pseudomonas aeruginosa* ATCC 27853 strains are 3.80 mg/mL, 3.80 mg/mL, 7.60 mg/mL, and 7.60 mg/mL, respectively. Based on these results, *Staphylococcus aureus* ATCC 25923 and *Acinetobacter*

*septicus* DSM 19415 are more sensitive to boric acid than *Escherichia coli* ATCC 35218 and *Pseudomonas aeruginosa* ATCC 27853. The MICs of borax obtained for *Staphylococcus aureus* ATCC 25923, *Acinetobacter septicus* DSM 19415, *Escherichia coli* ATCC 35218, and *Pseudomonas aeruginosa* ATCC 27853 strains are 23.80 mg/mL, 23.80 mg/mL, 47.60 mg/mL, and

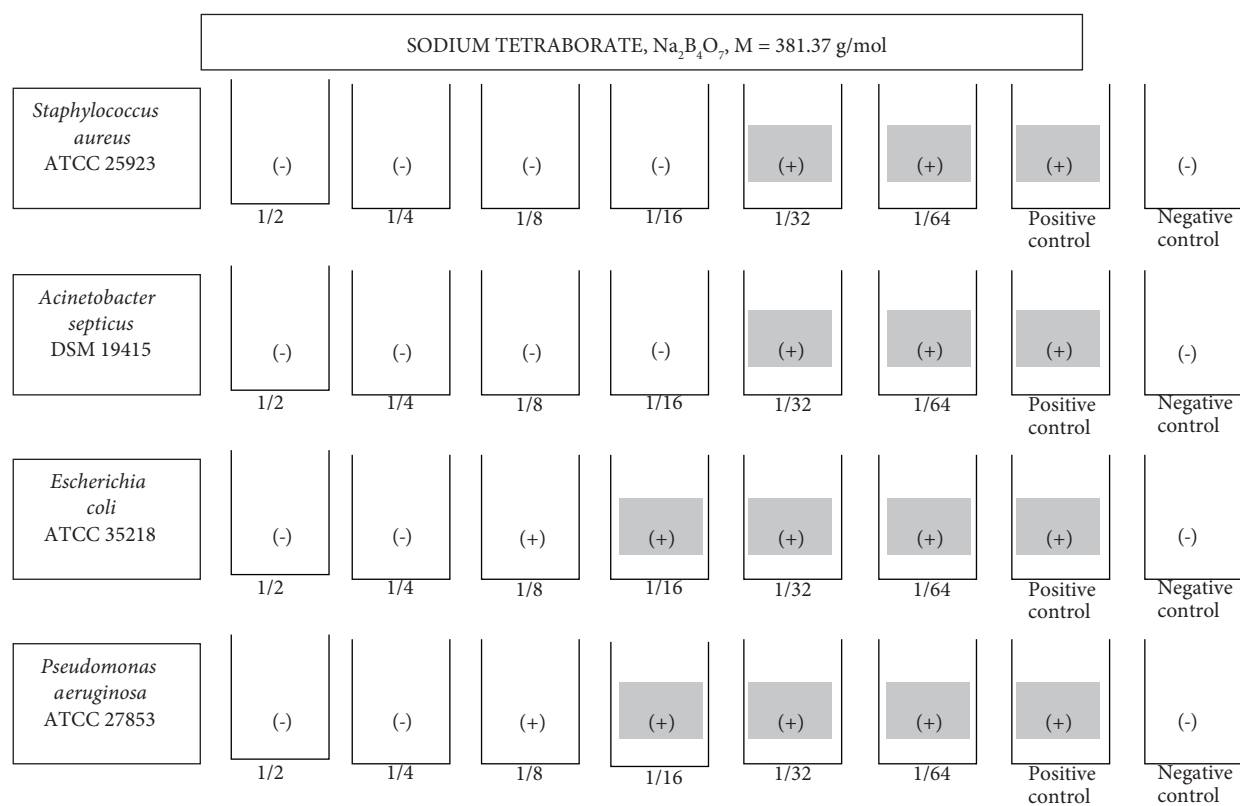


Figure 3. Minimum inhibitory concentrations of borax (sodium tetraborate).

47.60 mg/mL, respectively. *Escherichia coli* ATCC 35218 and *Pseudomonas aeruginosa* ATCC 27853 strains were thus found to be more resistant to borax than the *Staphylococcus aureus* ATCC 25923 and *Acinetobacter septicus* DSM 19415 strains.

All the corresponding pH values of boric acid and borax are given in Table 1. The pH value had no inhibitory effect on the bacteria from the evaluation of the sterile saline, for both boric acid and borax samples (Figure 4).

The MICs and MBCs for both boric acid and borax are given in Table 2. As shown in Table 2, the MICs and MBCs of boric acid and borax were similar.

## Discussion

Boron is an essential micronutrient for living organisms and has a narrow range in nature (18). Higher concentrations of boron show toxic effects with several mechanisms (4,5). Boron has a high affinity for ribose, a constituent of several essential

biological molecules of vitality such as ATP, NADH, NADPH, and RNA (6). Excessive boron impairs protein synthesis, causes mitochondrial dysfunction, and disrupts cell division and development (4). Boron is also involved in quorum sensing, which is a vital mechanism in microorganisms and is impaired by increased boron concentrations (6,7). Moreover, a variety of enzymes involved in stimulation, stabilization, or inhibition in microorganisms, plants, animals, and humans interact with boron compounds. Boron is required for the maintenance of the structure and functioning of cell membranes (3), and excess boron impairs the membrane function, integrity, conformation, and transport capacity (19–23).

Two distinct disciplines, environmental engineering and medicine, evaluate the detrimental effects of excess boron concentrations on living organisms for 2 different reasons. In environmental engineering, biological removal of excess boron from water is essential, as it is cost-effective and easier when compared to more complicated technologies (9). In

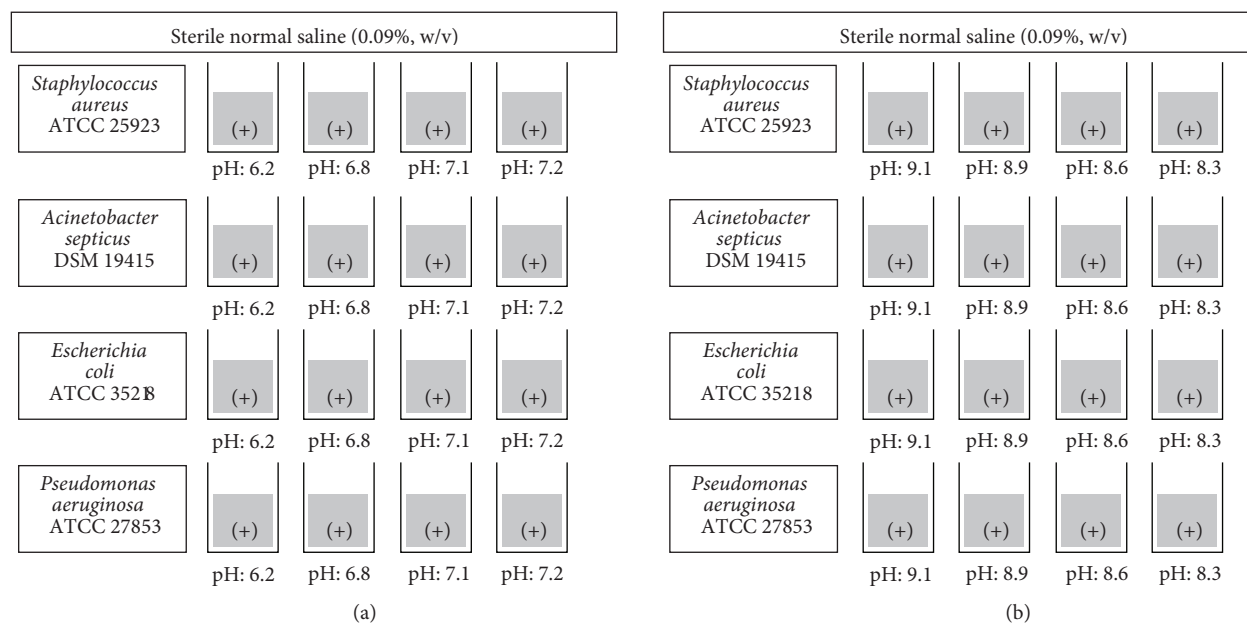


Figure 4. Effects of pH on growth of bacteria for a) boric acid and b) borax.

Table 1. pH values of the boric acid and borax.

Boric acid ( $H_3BO_3$ ), M = 61.83 g/mol			Sodium tetraborate ( $Na_2B_4O_7$ ), M = 381.37 g/mol		
Dilution	pH	Amount of boric acid (mg/mL)	Dilution	pH	Amount of sodium tetraborate (mg/mL)
1/2	6.21	30.5	1/2	9.15	190.6
1/4	6.78	15.25	1/4	8.93	95.3
1/8	7.10	7.60	1/8	8.60	47.6
1/16	7.20	3.80	1/16	8.30	23.8

Table 2. The results of MIC and MBC tests of boric acid and borax.

Microorganisms	Boric acid ( $H_3BO_3$ ), M = 61.83 g/mol		Sodium tetraborate ( $Na_2B_4O_7$ ), M = 381.37 g/mol	
	Minimum inhibitory concentration (MIC) (mg/mL)	Minimum bactericidal concentration (MBC) (mg/mL)	Minimum inhibitory concentration (MIC) (mg/mL)	Minimum bactericidal concentration (MBC) (mg/mL)
<i>Staphylococcus aureus</i> ATCC 25923	1/16 3.80 mg/mL	1/16 3.80 mg/mL	1/16 23.80 mg/mL	1/16 23.80 mg/mL
<i>Acinetobacter septicus</i> DSM 19415	1/16 3.80 mg/mL	1/16 3.80 mg/mL	1/16 23.80 mg/mL	1/16 23.80 mg/mL
<i>Escherichia coli</i> ATCC 35218	1/8 7.60 mg/mL	1/8 7.60 mg/mL	1/8 47.60 mg/mL	1/8 47.60 mg/mL
<i>Pseudomonas aeruginosa</i> ATCC 27853	1/8 7.60 mg/mL	1/8 7.60 mg/mL	1/8 47.60 mg/mL	1/8 47.60 mg/mL

medicine, the implementation of boron compounds in the antibiotic industry is important in order to overcome increasing resistance to antibiotics. Hence, the results of the present study are of the utmost important as they show the MIC and MBC values of common human pathogens, which are also used for biological removal of boron compounds from water. According to these results, both boric acid and borax have similar MIC and MBC values on standard *Staphylococcus aureus* ATCC 25923, *Acinetobacter septicus* DSM 19415, *Escherichia coli* ATCC 35218, and *Pseudomonas aeruginosa* ATCC 27853 strains.

Industrial wastewaters are usually transferred to domestic wastewater plants and refined when they exceed the limit of chemical oxygen demand (COD) or biological oxygen demand (BOD). Being used as the raw materials in several different industrial areas, boron compounds are also subjected to the same refinement procedures. However, biological removal of boron is thought to be inefficient, due to the toxic effects of high levels of boron compounds on living organisms (9). Therefore, advanced chemical removal procedures with higher operational and equipment prices are implemented to solve this problem. The microorganisms used in this study are the common bacteria of sewage systems. Therefore, no additional effort is needed to inoculate these bacteria in the wastewater refinement plants. This makes the study method a highly cost-effective and efficient procedure. The MIC and MBC values obtained for the boron compounds in this study are significant as they show the highest allowable concentrations of boron that do not prevent biological removal with these bacteria. According to the current literature, there have been no published data to show the MIC and MBC values of boron compounds. Overall, these results show that finding the MIC and MBC values

of boron compounds is also important aside from the COD and BOD parameters in the wastewater refinement processes.

There are ongoing efforts to use boron-containing products in several different areas of medicine from cancer therapeutics to oral antidiabetic, anticoagulants, and anti-infective drugs (11). As anti-infectives, the only current use of boronic compounds is in parasitic infections (10). However, there are several phase 1–3 studies on the use of boronic compounds as antiviral, antifungal, antituberculous, and antibacterial agents (11). Boronic acid derivatives can be effectively used to handle the problem of beta-lactamase resistance. Boron atoms mimic the carbon of the beta-lactam ring and selectively inhibit the serine protease family of beta-lactamases (24). Boronic esters also inhibit menaquinone methyl transferase, which can make them novel antibiotic agents for gram-positive bacteria (25). Therefore, the MIC and MBC values of borax and boric acid on *Staphylococcus aureus*, *Acinetobacter septicus*, *Escherichia coli*, and *Pseudomonas aeruginosa* strains are important, as these values can be used in future research for antibiotic development.

In conclusion, the results of the present study show that boric acid and borax have similar MIC and MBC values for some bacterial strains, which can be used for the biological removal of boron compounds from water. According to these results, water containing boron can be biologically treated unless it has reached specified boron values. These strains are also the common pathogens for human infectious diseases. Overall, these results are promising, not only for the improvement of biological removal techniques of boron compounds, but also for the implementation of boron compounds as antibiotics in medicine.

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