

# Alleviation of plant boron toxicity by using water to leach boron from soil contaminated by wastewater from rubber wood factories

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**ABSTRACT:** Wastewater from rubber wood furniture factories contains large amounts of boron (B) which contaminates the soil and causes unusual symptoms in plants in the area. We aim to establish the toxicity effects in tomato seedlings planted in Kho Hong (Kh) soil series irrigated with wastewater and compare them with seedlings grown in the same soil series but receiving tap-water containing B, S, Ca, or Na at the same concentrations as found in the wastewater. Then, we study B leaching from B-contaminated soil in a column by applying distilled water at different rates. We alleviate the B contamination in the Kh soil series by using water to leach out the B, then planting tomato seedlings. The results showed that in plants subjected to the wastewater or B treatments, growth was reduced and the plants showed tip and marginal chlorosis and necrosis of mature leaves at high B concentrations. The boron in B-contaminated soil was decreased to a greater degree by the application of a larger amounts of leaching water. Using 167.5 mm (field capacity) of water leached the B in the topsoil (0–15 cm) from a concentration of 11.11 mg/kg down to 2.83 mg/kg, a nontoxic level (< 3 mg/kg), and the leached B accumulated in the subsoil (15–30 and 30–50 cm). However, using 1000, 2000, or 4000 mm of water leached the B from the soil to below a toxic level with only a slight accumulation in the subsoil. This indicates that applying water to leach B from soil decreased the extractable B to below a toxic level, resulting in a decrease in plant B uptake. As a result, the tomato seedlings showed better growth in terms of height, leaf number, and plant dry weight than those of the control treatment.

**KEYWORDS:** boron toxicity symptoms, tip and marginal chlorosis, necrosis, extractable boron, leaching, rubber tree

## INTRODUCTION

Boron (B) is an essential plant micronutrient which can be phytotoxic when present in soil at high concentrations. Boron deficiency is of concern in areas receiving plentiful rainfall while B toxicity may be a problem in arid areas. The concentration range between plant deficiency and toxicity is narrow; therefore B contamination in soils may lead to plant toxicity. Most values of the B critical concentration for deficiency range from 0.15–0.5 mg B per kg of soil (extracted by hot water) and values greater than 3 mg B/kg are predictive of areas at risk of B toxicity<sup>1</sup>.

Boron toxicity limits plant growth on soils of arid and semi-arid environments throughout the world<sup>2</sup>. It has been noted that B toxicity is a widespread constraint to cereal and legume crops and pasture production in southern Australia. It occurs in extensive areas with annual rainfall below 400 mm. Elsewhere in the world, irrigation by high B water is the main

cause of B toxicity<sup>1</sup>. The occurrence of high B in soils derives from both natural and anthropogenic sources which are commonly associated with industry or irrigation practices. Citrus grown in South Florida, USA, contain excessive B due to an imbalance supply of this element in the reclaimed wastewater<sup>3</sup>. Soils derived from marine origin have the same potential problems as marine sediments containing very high B levels which have been the main source of B toxicity in many soils in Southern Australia<sup>4</sup>.

In spite of its agronomic importance, the way in which B causes toxicity is unknown. Sunflower leaves respond to conditions of B toxicity by producing large concentrations of cysteine and glutathione. This biosynthetic pathway is essential in the resistance and adaptability of plants to different type of stress. In addition, it has also been proposed that B toxicity impedes the conversion of cysteine to glutathione. External application of cysteine, and especially of glutathione, significantly reduces B toxicity<sup>5</sup>.

Tomato plants exhibit visible symptoms when grown in conditions that have excess B<sup>6</sup>. The first sign of B toxicity is a yellow-green interveinal chlorosis, which develops first on the oldest leaves and progresses to the youngest. Later, small patches of necrotic tissue appear between the minor veins and extend to the midribs. There is significant reduction of neither shoot nor root dry weight. Besides, tomato plants grown in high B contain decreased levels of superoxide dismutase, peroxidase, and polyphenol oxidase. In melons pretreated with a solution containing high B, fresh and dry plant weights decrease significantly with increasing B concentrations, and fruit set is completely inhibited at the highest B treatment (5.3 mM)<sup>7</sup>.

Most man-made sources of high B derive from industrial and waste effluents which often contain detergents with high levels of B. A common industrial source of high B is as a by-product of coal combustion in the form of both fly ash<sup>8</sup> and residue<sup>2</sup>. Boric acid and borate minerals are widely used in a number of industrial applications such as the manufacture of glass and porcelain and the production of leather and carpets, as well as in photographic chemicals and fertilizers. The main application of B is, however, the use of sodium perborate as an oxidation bleaching agent in domestic and industrial cleaning products<sup>2</sup>.

Rubber trees are an important economic crop in Thailand, especially in the South, and there are 433 rubber wood furniture factories in Thailand<sup>9</sup>. These factories use boric acid and borax as wood preservatives, resulting in high B in the wastewater. Unfortunately, when this wastewater contaminates the soil around the factories, this leads to unusual symptoms in the plants surrounding the factories<sup>10</sup>. Therefore, this study was conducted to establish the cause of these symptoms and to explore possible ways to alleviate the problem of B contaminated soils.

## MATERIALS AND METHODS

Experiments were conducted using Kho Hong soil series (Kh: Coarse-loamy, kaolinitic, isohyperthermic Typic Kandiodults), the most widely distributed soil in the south of Thailand. Tomato (*Lycopersicon esculentum*, Mill.) C.V. Srida, a B tolerant plant, was used as a test crop. Three experiments were conducted in a laboratory and at a greenhouse facilities as follows:

### Establishing the effects of toxicity

An experiment was conducted using a completely randomized design (CRD) with 4 replications and composed of 6 treatments: without wastewater (Control), 100 ml of wastewater (+WW), and using tap water

with added either boron (+B), sulphur (+S), calcium (+Ca), or sodium (+Na) in quantities appropriate to 100 ml of wastewater. The 30-day-tomato seedlings were transplanted in 5 kg of Kh soil in plastic bags containing sufficient nutrients<sup>11</sup>. Fifty days after being transplanted, the tomato plant dry weights were recorded and the B, Ca, S, and Na contents of the plants were analysed<sup>12</sup>.

### Leaching of boron in B-contaminated soil

A 15-cm-depth of B-contaminated soil (10 mg/kg) was arranged over a 35-cm-layer of uncontaminated soil (0.30 mg/kg) in a 3-inch-diameter column. An experiment was then conducted using a CRD with 3 replications consisting of 5 treatments as follows: leaching B by distilled water at rates of 0, 167.5 (field capacity), 1000, 2000 (annual rainfall), or 4000 mm. At the end of the experiment the soil column was dismantled and the soil at depths of 0–15, 15–30, and 30–50 cm were collected for B analysis<sup>13</sup>.

### Alleviation of B contamination by water leaching

An experiment was conducted using a CRD with 5 replications and 4 treatments as follows: application of tap water at rates of 0, 300, 600, or 900 mm. The 30-day-tomato seedlings were transplanted in 5 kg of Kh soil series containing sufficient nutrients<sup>11</sup>. Sixty days after being transplanted, plant growths (leaf number, dry weight, and height) were recorded and plant nutrients were analysed. At the end of the experiment, the soil in each treatment was sampled and prepared for analysis of its pH, electrical conductivity, extractable B, available N, and P, and exchangeable K<sup>12</sup>.

## RESULTS

### Establishing the effects of toxicity

It was found that applying 100 ml of wastewater (+WW) per pot significantly decreased the seedling growth, and caused the plants to die in the same way as in the +B treatment (Table 1). The plant dry weights in these treatments were 1.37 and 1.10 g/plant, respectively, whereas it was 2.37 g/plant in the control treatment. Further, the tomatoes in the +WW and +B treatment displayed marginal leaf necrosis with B contents of up to 1804 and 1867 mg/kg, respectively. However, the dry matter and B in the plants subjected to the +S, +Ca, or +Na treatments were not significantly different from those in the control treatment (85 mg/kg).

**Table 1** Effect of wastewater from wood rubber furniture factories on tomato plant dry weight and plant B, S, Ca, and Na concentration.

| Treatment | Dry weight<br>(g/plant) | Concentration (mg/kg) |      |      |                    |
|-----------|-------------------------|-----------------------|------|------|--------------------|
|           |                         | B                     | S    | Ca   | Na                 |
| Control   | 2.37 <sup>a</sup>       | 85 <sup>c</sup>       | 3427 | 4008 | 1042 <sup>bc</sup> |
| +WW       | 1.37 <sup>c</sup>       | 1804 <sup>a</sup>     | 4121 | 3943 | 1305 <sup>b</sup>  |
| +B        | 1.10 <sup>c</sup>       | 1867 <sup>a</sup>     | 4335 | 5205 | 1117 <sup>bc</sup> |
| +S        | 2.28 <sup>a</sup>       | 64 <sup>c</sup>       | 4316 | 5345 | 998 <sup>c</sup>   |
| +Ca       | 2.23 <sup>ab</sup>      | 59 <sup>c</sup>       | 5451 | 5283 | 1219 <sup>bc</sup> |
| +Na       | 1.64 <sup>ab</sup>      | 45 <sup>c</sup>       | 5185 | 5185 | 1607 <sup>a</sup>  |
| F-test    | **                      | **                    | NS   | NS   | **                 |
| C.V. (%)  | 22                      | 37                    | 23   | 19   | 14                 |

The same letter in the columns means a non-significant difference at 95% by DMRT.

NS=not different, \*\*=significant difference at 99%.

### Leaching of B in B-contaminated soil

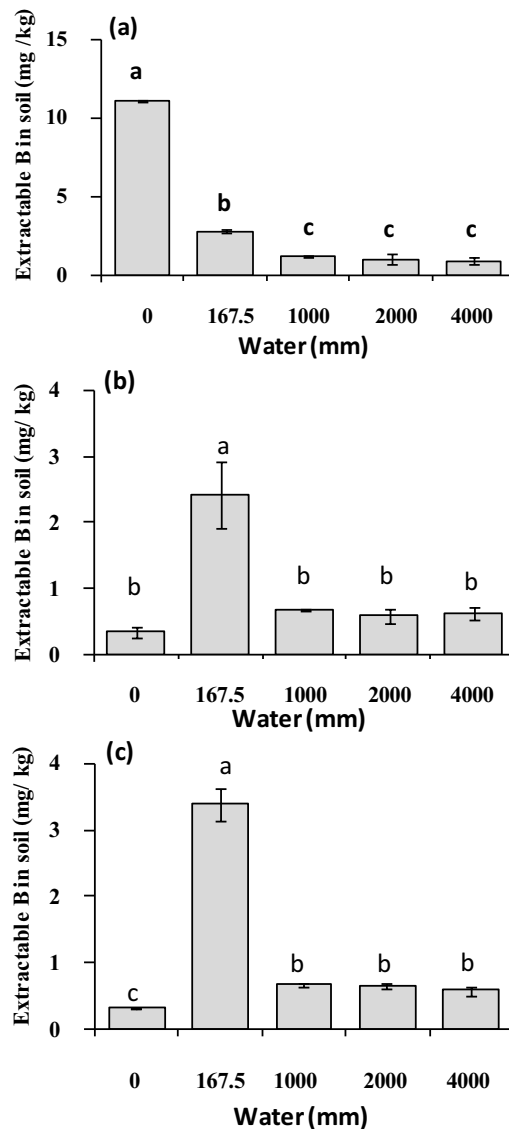
Boron concentrations in the soil decreased as the rate of water application increased (Fig. 1). The application of 167.5 mm of water leached B from the soil at depths of 0–15 cm reducing the concentration from 11.11 mg/kg to 2.83 mg/kg. However, the B leached down to accumulate in lower soil depths (> 15 cm). Using 1000, 2000, or 4000 mm of water leached the B from the soil at depths of 0–15 cm down to concentrations of 1.21, 1.24, and 0.93 mg/kg, respectively, with only slight accumulations at lower depths.

### Alleviation of B contamination in soil by water leaching

The results indicated that using water at levels of 300, 600, and 900 mm to leach boron from the soil decreased the extractable B (Table 2) and therefore the tomato seedlings showed better growth than those of the control treatment (Fig. 2 and Fig. 3). It was also found that using 600 mm of water resulted in the optimal growth of the tomatoes in terms of leaf number, dry weight, and height. The concentration of B in the plants decreased markedly with increasing rates of application of leaching water (Fig. 4). It can also be seen that the extractable B in the soil sharply decreased with increases in the water leaching rate (Table 2). The soil pH, electrical conductivity, and exchangeable K also decreased.

### DISCUSSION

Boron toxicity in plants is uncommon unless B has been added to the plants environment<sup>14</sup>. Waste-



**Fig. 1** Extractable B in soil at depths of (a) 0–15 cm, (b) 15–30 cm, and (c) 30–50 cm after leaching by different rates of water. The same letter over the bars means a non-significant difference at 95% by DMRT.

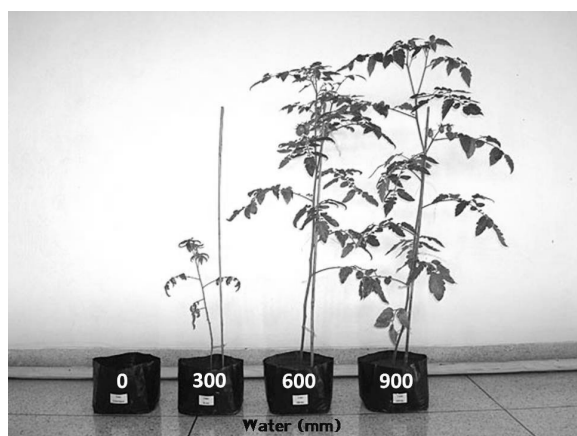
water from rubber wood furniture factories contains 1000–1500 mg/l B as boric acid ( $H_3BO_3$ ) and borax ( $Na_2B_4O_7 \cdot 10H_2O$ ) are used in the wood preservation process<sup>15</sup>. When soils become contaminated with B, plants grown surrounding the factories such as rubber trees (*Hevea brasiliensis*), lonkong trees (*Aglaia dookoo* Griff.), bananas (*Musa sapientum* Linn.), and coconuts (*Cocos nucifera* Linn.) are affected. These plants show necrosis at the tip and margin of lower leaves<sup>10</sup>. These symptoms are consistent with the typical symptoms of B toxicity occurring on mature

**Table 2** Some chemical properties of, and nutrients in B contaminated soils after leaching by different rates of water application.

| Water application | pH<br>(soil:water=1:5) | EC (dS/m)<br>(soil:water=1:5) | Extr. B<br>(mg/kg) | NH <sub>4</sub> <sup>+</sup> + NO <sub>3</sub> <sup>-</sup><br>(mg/kg) | Avai. P<br>(mg/kg) | Exch. K<br>(cmol <sub>c</sub> /kg) |
|-------------------|------------------------|-------------------------------|--------------------|--|--------------------|------------------------------------|
| 0 mm              | 7.48 <sup>a</sup>      | 0.23 <sup>a</sup>             | 96.15 <sup>a</sup> | 41.0 <sup>ab</sup>   | 27.6               | 0.43 <sup>a</sup>                  |
| 300 mm            | 7.36 <sup>a</sup>      | 0.13 <sup>b</sup>             | 7.10 <sup>b</sup>  | 49.6 <sup>a</sup>  | 27.5               | 0.37 <sup>b</sup>                  |
| 600 mm            | 6.91 <sup>b</sup>      | 0.13 <sup>b</sup>             | 3.00 <sup>b</sup>  | 41.3 <sup>ab</sup>   | 26.0               | 0.35 <sup>c</sup>                  |
| 900 mm            | 6.87 <sup>b</sup>      | 0.10 <sup>c</sup>             | 1.06 <sup>b</sup>  | 37.2 <sup>b</sup>  | 25.5               | 0.34 <sup>c</sup>                  |
| F-test            | **                     | **                            | **                 | *  | NS                 | **                                 |
| C.V. (%)          | 1.3                    | 7.5                           | 24.7               | 14.6   | 8.4                | 3.5                                |

The same letter in the columns means a non-significant difference at 95% by DMRT.

NS=not different, \* and \*\*=significant difference at 95 and 99%, respectively.



**Fig. 2** Growth of tomato plants grown in B contaminated soil after leaching by different rates of water application (the plant in the control treatment died within 5 days of being transplanted).

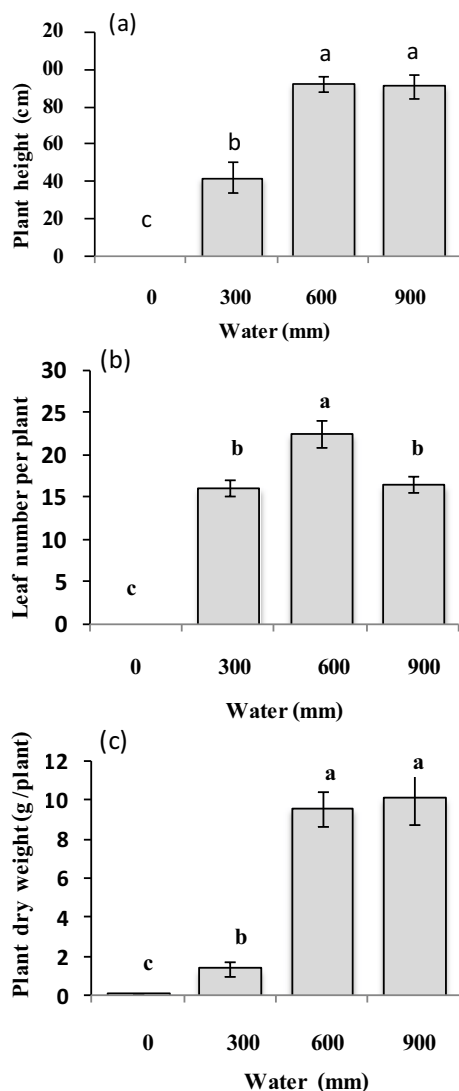
leaves, showing marginal or tip chlorosis (or both) and necrosis<sup>6,16</sup>.

Results from our experiment showed that tomato growth in the treatments which received wastewater (+WW) and boron (+B) was markedly suppressed, and B concentration in plants was significantly higher (Table 1). The results clearly confirmed that the main cause of the plant symptoms was B toxicity caused by receiving high amounts of wastewater or B directly. It has also been reported that applying high amounts of wastewater from rubber wood factories decreases tomato growth, causing chlorosis of leaf tips and the margins of old leaves, followed by necrosis and finally plant death. However, applying small amounts of wastewater tended to increase the growth of the tomato plants<sup>10</sup>. This finding is supported by research showing that 30-day-tomato seedlings died

5 days after being transplanted into soil containing high B (165–195 mg/kg) caused by contamination by wastewater from rubber wood factories<sup>17</sup>.

Because of the high leaching rate, B is rarely accumulated in soil especially in areas receiving plentiful rainfall<sup>1,18</sup>. The alleviation of B concentrations in soils contaminated with B from fly ash by leaching has been found to be feasible<sup>19</sup> and the application of leaching by water can decrease B in the top soil significantly (Fig. 1a). Although using 167.5 mm of water decreased the level of B in the top soil, B accumulated in the sub soil (Fig. 1b and c). However, the application of 1000 mm of water to leach B from B-contaminated soil reduced B at the 0–15 cm depth from 11.11 mg/kg to 1.21 mg/kg, a nontoxic level (< 3 mg/kg) as reported<sup>1</sup>, and resulted in only slight B accumulations at the 15–50 cm depth (Fig. 1). Using over 1000 mm of water also notably reduced the concentration of B, thus suggesting that B in contaminated soil can be leached by rainwater within a year in areas where there is an annual rainfall of 1000 mm or more.

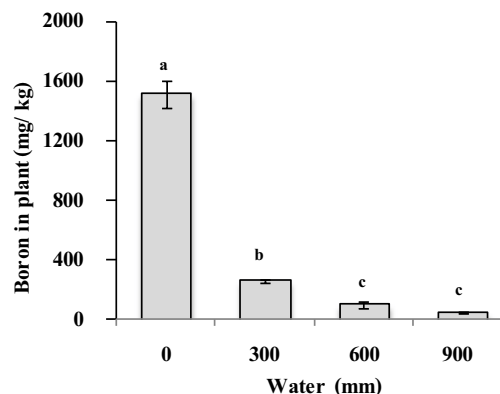
As for using water to leach B from B-contaminated soil from rubber wood factories, the results of this study indicate that using water decreased the extractable B (Table 2) and therefore the tomato seedlings showed better growth than those in the control treatment (Fig. 2 and Fig. 3). It was found that using 600 mm of water resulted in the optimal growth of the tomato plants. This can be explained as the levels of extractable B (Table 2) being reduced to a nontoxic level (< 3 mg/kg). It can be observed that the concentration of B in the plants decreased markedly from 1510 mg/kg in the control treatment to 100 mg/kg in the 600 mm treatment (Fig. 4). If the B concentration is over 100 mg/kg, it will be toxic to plants<sup>20</sup>. These results support the finding that B deficiency is of concern in areas receiving plentiful



**Fig. 3** (a) Plant height, (b) leaf number, and (c) plant dry weight of tomato plants grown in B contaminated soil after leaching by different rates of water. The same letter over the bars means a non-significant difference at 95% by DMRT.

rainfall while B toxicity may be a problem in arid areas<sup>1</sup>. It has also been noted that B added to soils remains soluble and up to 85% can be leached in sandy soils containing low amounts of organic matter<sup>14</sup>.

The current results confirm that wastewater, derived from rubber wood furniture factories, containing high levels of B can cause plant B toxicity. However, when soil is contaminated with B, the B can be leaching out by applying water and this is a simple and effective treatment. It is possible that rainwater can leach B from contaminated soil down to a nontoxic level within a year, especially in areas with an annual rainfall of 1000 mm or more.



**Fig. 4** Boron in plant grown in B contaminated soil after leaching by different rates of water. The same letter over the bars means a non-significant difference at 95% by DMRT.

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