



## Effects of Elemental Sulphur Application Rates on Soil Al<sup>3+</sup> Solubility and Its Concentration in Maize Plants (*Zea mays L.*)

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### ABSTRACT

A greenhouse experiment was conducted to elucidate the influence of soil acidification due to application of different doses of elemental sulphur (0, 0.5, 1.0 and 2.0 g S kg<sup>-1</sup> soil) on Al<sup>3+</sup> solubility at 0, 20 and 40 days after incubation. Maize plants were grown after soil treatment with the elemental S and were allowed to grow for 45 days. The results showed that addition of elemental sulphur significantly increased the soil acidity; each g S decreased soil pH for 1.52 units. The Al<sup>3+</sup> concentration in soil remained rather unchanged from the pH value of 7.3 to around 5 and experienced a 22000-time increase at the pH value of 4. Soil acidification from the background of 7.03 to 6.29 resulted in 41.83% increase in root Al<sup>3+</sup> concentration and it was not significantly affected with further soil acidification. Soil acidification progressively decreased Al<sup>3+</sup> concentration in the stem but it failed to affect Al<sup>3+</sup> concentration in the leaves. The optimum rate of sulphur for maize without the risk of Al<sup>3+</sup> toxicity under greenhouse conditions was 0.82 g S kg<sup>-1</sup> soil. Further evaluation under field conditions is required.

*Keywords:* Soil acidification, Al<sup>3+</sup> toxicity, maize growth

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### INTRODUCTION

The high pH soils that are mainly located in arid regions amount to about 50 mil km<sup>2</sup> or more than 30% of world soils (Manahan, 2004; Shenker et al., 2005). In addition, these soils can be found in several isolated areas, especially in regions near limestone hills, which occur widely in Malaysia (Tan, 2002). Plant growth is usually restricted

in high pH soils because of the limited availability of essential nutrients (Lindsay, 1979; Shenker et al., 2005; Wang et al., 2006). Therefore, acidification of high pH soils is recommended to enhance nutrient availability and to improve plant growth (Ye et al., 2011).

Elemental sulphur is of special interest in increasing soil nutrient mobility (Jankowski et al., 2015; Sienkiewicz-Cholewa & Kieloc, 2015) as it is slow-release acidifying and is commonly available (Chien et al., 2011). The acidifying function of S originates from its microbial oxidation to sulphuric acid over time (Vidyalakshmi et al., 2009). However, according to some authors, application of elemental sulphur in soil amendment in their studies did not show significant change in soil chemical properties such as acidity and nutrient availability (Sameni et al., 2004; Shenker & Chen, 2005; Skwierawska et al., 2012). This might be due to both unsuccessful oxidation of applied sulphur as well as high carbonate content of the soil. However, successful oxidation of elemental sulphur and a significant change in soil chemical properties and nutrient availability are well documented for some soils (Ye et al., 2010; Khalid et al., 2012). Nonetheless, the response of nutrients to soil acidification is difficult to predict. In addition, their interaction affects their availability to crops as an over-abundance of one nutrient may result in a deficiency of another. For instance, soil acidification may increase Al solubility in soil; Al is not considered an

essential nutrient (Meriño-Gergichevich et al., 2010). Among all available Al species trivalent aluminum ( $Al^{3+}$ ) is the most toxic to plants and its concentration enhances in acidic conditions. Al-toxicity results in alterations of the physiological and biochemical processes of plants and consequently their productivity. The decrease in root growth is one of the initial and most evident symptoms of Al-toxicity at micro-molar concentrations in plants (Meriño-Gergichevich et al., 2010).

The present study was conducted to elucidate the effect of elemental S on  $Al^{3+}$  concentrations in the Bintang Series of soil and its effect on maize growth. In addition, the effect of elemental S on  $Al^{3+}$  accumulation in the root and the above-ground parts of maize plants in greenhouse conditions was also studied.

## MATERIALS AND METHOD

### Experimental Site

Soil samples were collected from the A horizon (0-20 cm) of the Bintang Series of soil located in Perlis, Malaysia ( $6^{\circ} 31' 01.61''$  N and  $100^{\circ} 10' 12.43''$  E). The area, Bukit Bintang, is affected by limestone parent material and is host to natural vegetation (Karimizarchi et al., 2014). The experiment was conducted in greenhouse conditions at University Putra Malaysia (UPM).

### Planting and Cultural Practices

Sweet maize (*Zea mays* L.) seeds, Masmadu, were collected from the

Malaysian Agricultural and Development Research Institute (MARDI). The seeds were germinated in laboratory conditions and transplanted to plastic pots (30 cm diameter and 50 cm height). Each pot contained 10 kg soil and held three plants, which were thinned to one within one week. The seedlings were grown for 45 days in greenhouse conditions. The pots were weighed and irrigated daily to maintain 90% soil field capacity moisture content. Fertiliser was applied according to MARDI's recommendation: 120 kg N/ha in the form of urea, 60 kg/ha P<sub>2</sub>O<sub>5</sub> in the form of triple superphosphate and 40 kg/ha K<sub>2</sub>O in the form of muriate of potash (Karimizarchi et al., 2014).

### Experimental Design and Treatment

The experiment was conducted in a completely randomised design (CRD) with four replications. There were 12 treatments including four levels of sulphur (0, 0.5, 1 and 2 g S per kg of soil) and three sampling times (0, 20 and 40 days) before the planting of maize.

### Plant and Soil Sampling and Analysis

Soil samples were collected before the maize planting and after harvest. The soil samples were air dried and ground (<2 mm) before use. Soil electrical conductivity and pH were measured in a soil-water suspension (10 g soil to 25 ml deionised water) 24 hours after shaking for 30 min on a reciprocal shaker. Total carbon, nitrogen and sulphur was determined using

a CHNS LECO analyser. Soil mechanical analysis was done using the pipette method (Gee et al., 1986) and textural class was determined by United States Department of Agriculture (USDA) soil textural triangle. Available Al<sup>3+</sup> concentration in the soil was extracted using the un-buffered and neutral extracting solution of CaCl<sub>2</sub> (Jones, 2001; Ye et al., 2011).

The maize plants were harvested after 45 days. Plant leaf, shoot and root tissue was separately washed in deionised water, then dried at 65°C and weighed. After grinding, weighed plant tissue was ashed in a muffle furnace at 480°C for about 10 h and dissolved in a diluted acid mixture (Jones, 2001). Al concentration was determined by ICP-OES (Perkin Elmer, Optima 8300).

### Statistical Analysis

To model the relationship between plant and soil properties, data collected were subjected to different regression models at the probability level of 0.05 with the help of the Sigmaplot software. The analysis of variance for different parameters was done following ANOVA technique. When F was significant at  $p \leq 0.05$  level, treatment means were separated using DMRT. Data were analysed following standard procedure using SAS software (version 9.1).

## RESULTS AND DISCUSSION

### Physicochemical Properties of Bintang Series Soil

The physicochemical characteristics of the Bintang Series soil are presented in

Table 1. Being silt loam in texture, the soil was found to be slightly alkaline in nature (pH=7.3) as it was affected by limestone parent material from the nearby hills. Base saturation was high, (56%); however, the calcium carbonate content of the soil

was not detected. Low calcium carbonate content that could be attributed to the high precipitation of the area, implied that the soil buffering capacity was low and did not need a high amount of acidic soil amendment such as elemental sulphur to reduce soil pH.

Table 1  
Soil physico-chemical properties of Bintang series soil

Soil property	Unit	Value or Concentration	Soil property	Unit	Value or Concentration
pH	-	7.30	Sand	%	9.00
CaCO <sub>3</sub>	%	Trace	Silt	%	66.40
C	%	1.75	Clay	%	24.60
N	%	0.12	Texture	-	Silt loam
S	%	0.004	FC	%	20.00
C/N	-	14.58	CEC	Cmol <sub>+</sub> kg <sup>-1</sup> soil	11.50
C/S	-	437.50	BS	%	56.00

**Effect of Elemental Sulphur on Soil Acidity**

Significant negative linear regression (P<1%) was found between soil pH and sulphur application rate (Figure 1), while increasing S rate soil pH decreased from the initial value of around 7.03 to 6.29, 5.26 and 3.94 at sulphur application rates of 0.5, 1 and 2 g kg<sup>-1</sup>, respectively. The regression

line slopes downwards with a slope of -1.52, which is consistent with the negative relationship anticipated between S rate and soil pH (Shenker & Chen 2005; Cui et al., 2004; Vidyalakshmi et al., 2009). This result reflected the successful oxidation of elemental sulphur in the Bintang Series soil and may have affected the availability of nutrients in the soil.

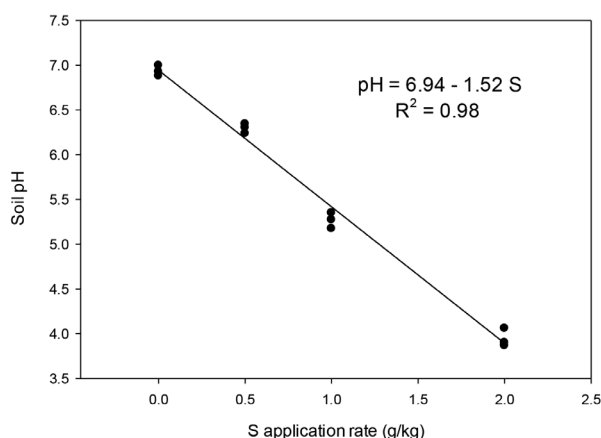


Figure 1. Effect of elemental sulphur application rates on Bintang Series soil pH

### Effect of Elemental Sulphur Application Rate on Soil Extractable Al<sup>3+</sup>

Sulphur application rate had a significant effect on Al<sup>3+</sup> solubility in the Bintang Series soil (Figure 2). Application of 2 g S kg<sup>-1</sup> of soil increased extractable Al<sup>3+</sup> from the background of 0.001 mg kg<sup>-1</sup> prior to S application to 21.78 mg kg<sup>-1</sup> after 40 days and tended to level off thereafter. Application of elemental sulphur up to 1 g S kg<sup>-1</sup> soil did not affect Al<sup>3+</sup> solubility. The low concentration of Al<sup>3+</sup> at the first three sulphur application rates was in line with the findings of Meriño-Gergichevich et al. (2010) i.e. most of the Al was bound to

insoluble forms such as aluminosilicates or precipitated as Al hydroxide. They reported that Al was solubilised from silicates and oxides to Al<sup>3+</sup> under low pH conditions. They also reported that there were various Al forms in soil and that their concentration depended on the degree and duration of Al compound hydrolysis. In addition, they found a significant correlation between low pH and high concentrations of phytotoxic Al species, which is related to the reduction of exchangeable bases in the soil solution. Below is the explanation of the relationship between soil Al<sup>3+</sup> and soil pH in conditions of our experiment as we discovered.

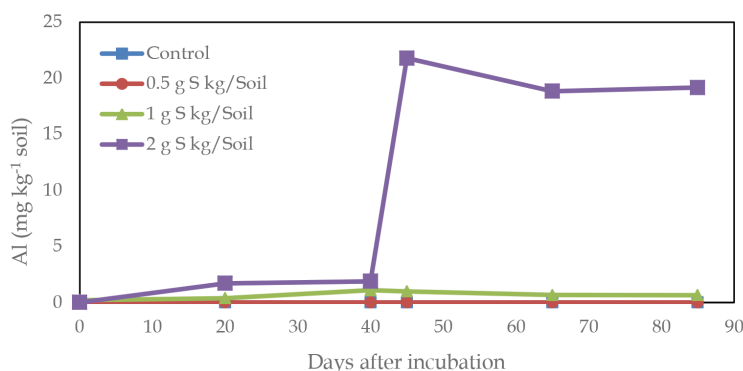


Figure 2. Effect of elemental sulphur on Al<sup>3+</sup> concentration at different incubation times

### Relationship Between Soil Al<sup>3+</sup> Concentration and Soil pH

Bintang Series soil acidification significantly increased soil Al<sup>3+</sup> solubility (Figure 3). The relationship between soil pH and Al<sup>3+</sup> concentration was nonlinear,  $Al = \frac{22.0}{\left(1 + \left(\frac{pH}{4.3}\right)^{16.94}\right)}$ ,  $R^2 = 0.94^{**}$  and soil Al<sup>3+</sup> concentration failed to change from a pH value of 7 to 5, after which a sharp increase

in Al<sup>3+</sup> concentration was observed. This was in line with the data presented by Franz et al. (2007), Hesterbeg et al. (1993) and Ward et al. (2011). They reported the release of plant-available Al at pH ≤ 5.5. In addition, McBride (1994) revealed that once soil pH is lowered to below 5.5, aluminosilicate clay and Al hydroxide minerals begin to dissolve, releasing Al-hydroxyl cations. Al<sup>3+</sup> will then exchange

with other cations from soil colloids, resulting in a build-up of  $Al^{3+}$  concentration in soil solution. As stated by Lambers et al. (2008) and Viani et al. (2014), the increase in weathering rate, the change in oxidation state of some nutrients and the displacement of cations from exchangeable sites due to high concentration of hydrogen ions accounted for the increases in soil nutrient mobility.

It was noted that the nonlinear relationship between soil pH and  $Al^{3+}$  concentration can be linearised if the relationship between the log of  $Al^{3+}$  concentration as a function of soil pH is considered,  $\log Al = 7.5 - 1.56 \text{ pH}$ ,  $R^2 = 0.92^{**}$ . Using the solubility equation of Gibbsite ( $pAl = 3pH - 8.5$ ), with 1 unit decrease in soil pH, Al solubility increases  $10^3$  times; in the conditions of our experiment, it increased  $10^{1.56}$  times.

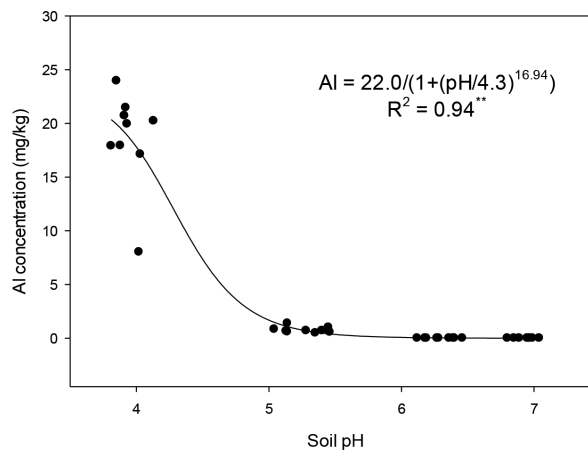


Figure 3. Effect of soil pH on  $Al^{3+}$  concentration in Bintang Series soil

### Effect of Elemental Sulphur on Maize Growth

The relationship between sulphur application rates and maize dry weight was quadratic in nature (Figure 4). Maize dry weight increased as elemental sulphur rate increased up to  $0.82 \text{ g kg}^{-1}$  soil, after which there was a sharp decrease in maize dry weight. This was mainly due to the significant increase in soil Mn and Zn availability (Karimizarchi & Aminuddin, 2015) and uptake by maize as demonstrated by Karimizarchi et al. (2014b). In addition,

our results showed that an addition of  $2 \text{ g S kg}^{-1}$  significantly decreased total dry weight of maize by 38.34% compared to the control (Figure 4). Although this reduction in maize biomass was related to the Mn and Zn toxicity (Karimizarchi et al., 2014b), the contribution of  $Al^{3+}$  toxicity remained unknown. Therefore, the effect of elemental sulphur on  $Al^{3+}$  concentration in different parts of maize became clear. At the same time, we also studied the relationship between  $Al^{3+}$  concentration in maize and growth.

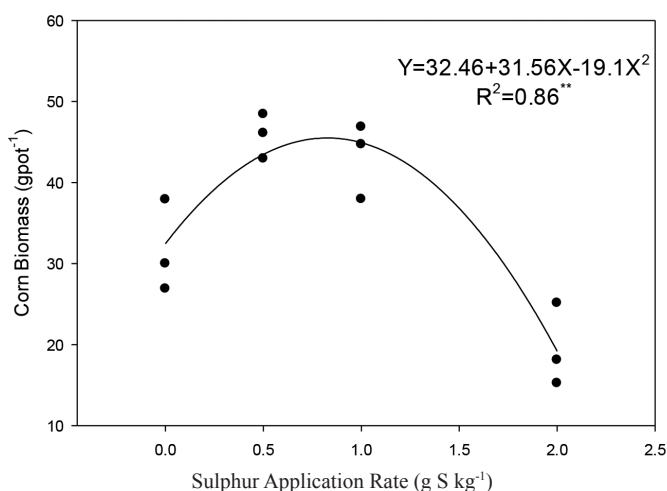


Figure 4. Effect of elemental sulphur application rate on maize dry weight

**Relationship between Leaf Al<sup>3+</sup> Concentration and Leaf Dry Weight**

There was a linear relationship between leaf biomass and leaf Al<sup>3+</sup> concentration (Figure 5). With one unit increase in leaf Al<sup>3+</sup> concentration, leaf dry matter decreased by 8.49 g per pot. Although this was in line with the generally accepted theory that Al<sup>3+</sup> is not considered an essential nutrient (Meriño-Gergichevich et al., 2010), the coefficient of determination for this relationship was low

i.e. 0.5. In other words, Al<sup>3+</sup> concentration in the leaves explained only 50% of the variation in leaf performance; other nutrients may have had a crucial effect on the maize growth. This conclusion was supported by the fact that Al<sup>3+</sup> concentration in the leaves was far below the phytotoxicity level of Al<sup>3+</sup>, 13 µg g<sup>-1</sup> (Lidon et al., 2002). Karimizarchi et al. (2014b) also reported the toxicity of Mn and Zn in maize plants treated with 2 g S kg<sup>-1</sup> soil.

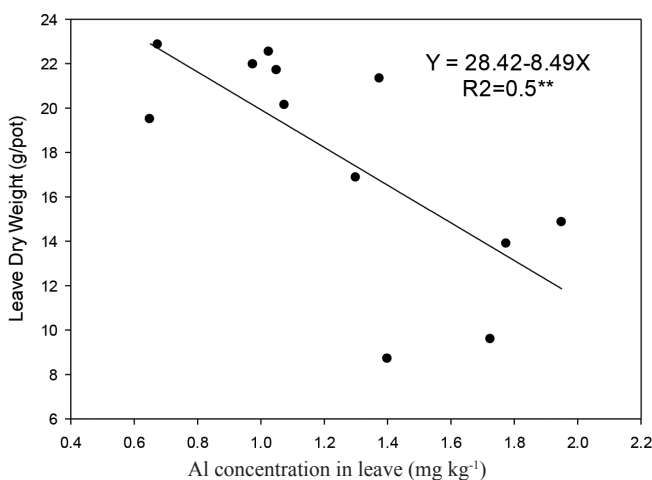


Figure 5. Relationship between Al<sup>3+</sup> concentration and dry weight of maize leaf

### Relationship between Root Al<sup>3+</sup> Concentration and Root Dry Weight

The relationship between Al<sup>3+</sup> concentration in root and root dry weight was quadratic in nature (Figure 6). Root dry weight increased as root Al<sup>3+</sup> concentration increased up to 187.5 mg kg<sup>-1</sup>, after which there was a slight decrease in root dry weight. This observation may signify the beneficial role of Al<sup>3+</sup> on maize root growth. In line with our finding, Lee (2013) and Barker et al. (2007) also found a beneficial effect of low levels of aluminum on root and shoot growth of non-accumulator plants such as soybean and maize. However, their findings are contrary with the general belief that the specific biological functions of Al<sup>3+</sup> for plants are unknown and this mineral is not regarded as an essential plant nutrient (Meriño-Gergichevich et al., 2010; Fernandes et al., 2013). The nontoxic

high concentration of Al<sup>3+</sup> in maize root in the conditions of our experiment can be related to the detoxifying role of sulphate (Meriño-Gergichevich et al., 2010; Robson, 2012; Karimizarchi et al., 2015), phosphate (Bennet et al., 1986; Robson, 2012), organic acid release by plants (Feng et al., 2001) and phosphorous deficiency (Bennet et al., 1986; Ward et al., 2011 & Karimizarchi et al., 2016). The relationship between sulphur application rate and Al<sup>3+</sup> concentration in the roots is another confirmation of the nontoxic effect of Al<sup>3+</sup> in maize in conditions of our experiment (Figure 7).

The maximum Al<sup>3+</sup> concentration in root was found to be 0.5 mg kg<sup>-1</sup> S, where maximum yield was obtained (Figure 4) but not in maximum S rate where the minimum plant growth (Figure 4) and maximum Al<sup>3+</sup> concentration in soil (Figure 2) was achieved.

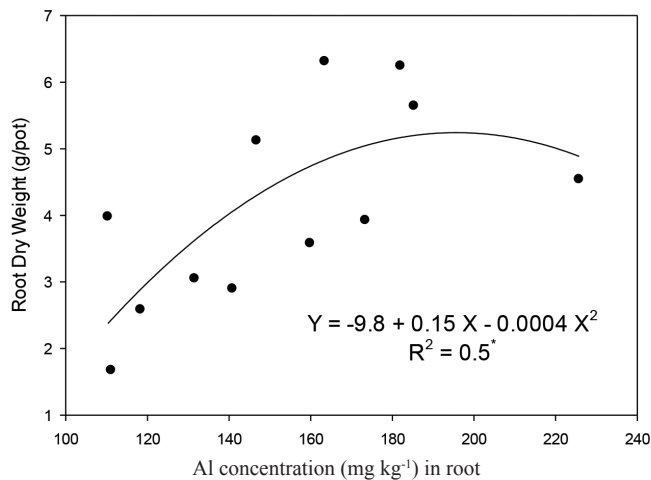


Figure 6. Relationship between Al<sup>3+</sup> concentration and dry weight of maize



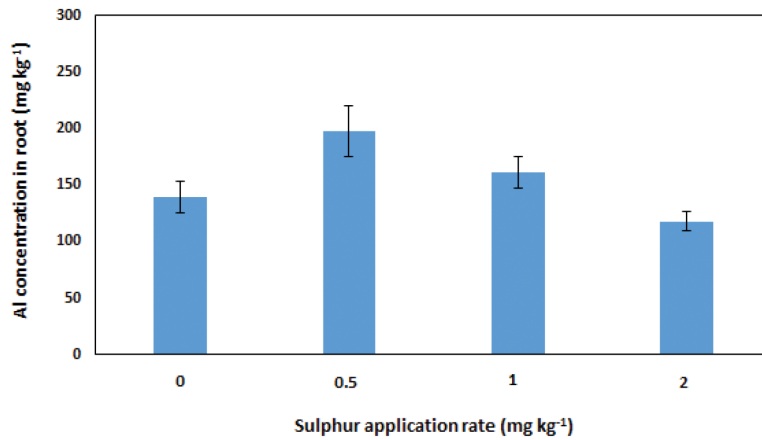


Figure 7. Effect of elemental sulphur rate on root Al<sup>3+</sup> concentration (mg kg<sup>-1</sup>). Bars show standard errors

### Relationship between Stem Al<sup>3+</sup> Concentration and Stem Dry Weight

The insignificant relationship between Al<sup>3+</sup> in maize stem and stem performance (data not shown) is another confirmation of the nontoxic effect of Al<sup>3+</sup> in maize in the conditions of our experiment. This

conclusion is further supported by the decreasing trend in Al<sup>3+</sup> concentration in the stem as a function of elemental sulphur application rate (Figure 8) and signifies the Al<sup>3+</sup> exclusion ability of maize as stated by Taylor (1988) and Kochian (1995).

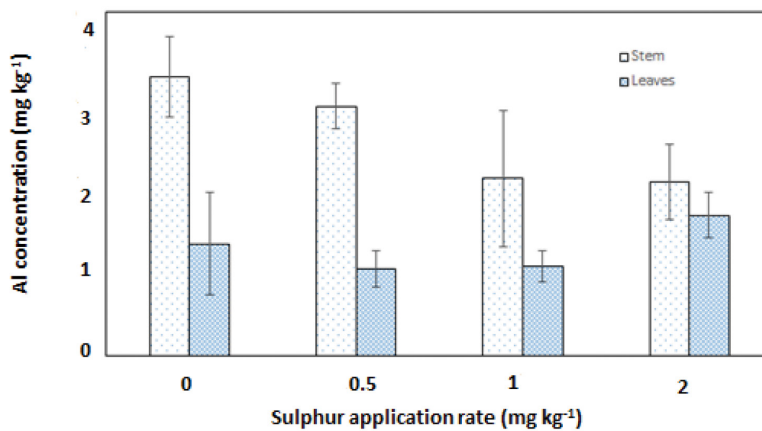


Figure 8. Effect of elemental sulphur application rate on Al<sup>3+</sup> concentration in maize leaf and stem. Bars show standard errors

### Al<sup>3+</sup> is Immobile Inside the Maize Plant

The distribution of Al<sup>3+</sup> in different parts of the maize plants grown in the conditions of our experiment signified the ability of maize to prevent Al<sup>3+</sup> transfer from root to stem and leaves. Al<sup>3+</sup> concentration in stem ranged from 2.02 to 3.24 mg kg<sup>-1</sup> (Figure 8) and was considered very low compared with the concentration in the root, where it ranged from 110 to 225 mg kg<sup>-1</sup> (Figure 7). Our data also demonstrated the lower Al<sup>3+</sup> concentration in maize leaves (varied from 1 to 1.63 mg kg<sup>-1</sup>) than in the stem. In addition, the low ratio of Al<sup>3+</sup> concentration in maize leaves and stem to root, 0.008 and 0.016 respectively, demonstrated that Al<sup>3+</sup> is an immobile nutrient in maize. This is in line with Barker and Pilbeam's (2007) finding. They reported a greater value of Al<sup>3+</sup> concentration in the roots than in the young leaves of maize.

### CONCLUSION

In this study, application of elemental sulphur decreased pH of the Bintang Series soil in linear trend. However, soil Al<sup>3+</sup> solubility was not significantly affected unless soil acidity dropped to a pH of around 4, the pH of hydrous oxide precipitation, where application of 2 g S kg<sup>-1</sup> soil increased the CaCl<sub>2</sub> extractable Al<sup>3+</sup>. In addition, the 22000-time increase in soil Al<sup>3+</sup> solubility due to application of 2 g S kg<sup>-1</sup> soil failed to increase Al<sup>3+</sup> concentration in maize tissue. The optimum rate of sulphur for maize production in greenhouse conditions was 0.82 g S kg<sup>-1</sup>

soil. Al<sup>3+</sup> toxicity was not implicated in the significant decrease in maize growth at sulphur rate of 2 g S kg<sup>-1</sup> soil.

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