



## Research article

Bioaugmentation of *Vibrio alginolyticus* in phytoremediation of aluminium-contaminated soil using *Scirpus grossus* and *Thypha angustifolia*Ipung Fitri Purwanti<sup>a</sup>, Adriana Obenu<sup>a</sup>, Bieby Voijant Tangahu<sup>a</sup>, Setyo Budi Kurniawan<sup>b</sup>, Muhammad Fauzul Imron<sup>c,\*</sup>, Siti Rozaimah Sheikh Abdullah<sup>b</sup><sup>a</sup> Department of Environmental Engineering, Faculty of Civil, Planning, and Geo Engineering, Institut Teknologi Sepuluh Nopember, Kampus ITS Sukolilo, Surabaya 60111, Indonesia<sup>b</sup> Department of Chemical and Process Engineering, Faculty of Engineering and Built Environment, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia<sup>c</sup> Study Program of Environmental Engineering, Department of Biology, Faculty of Science and Technology, Universitas Airlangga, Kampus C UNAIR, Jalan Mulyorejo, Surabaya 60115, Indonesia

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## ABSTRACT

This research analyses the performance of bacteria-assisted phytoremediation of aluminium (Al)-contaminated soil using native Indonesian plants namely, *Scirpus grossus* and *Thypha angustifolia*. A range finding test (RFT) was carried out for 14 days to obtain the tolerable Al concentration for both plants. A total of 2% and 5% (v/v) of *Vibrio alginolyticus* were bioaugmented during the 28-day phytoremediation test to enhance the overall Al removal. Result of the RFT showed that both plants can tolerate up to 500 mg/kg Al concentration. The addition of *V. alginolyticus* to the reactors resulted in a significant increment of Al removal from the contaminated soil ( $p < 0.05$ ). Such addition of *V. alginolyticus* increased the Al removal by up to 14.0% compared with that without-bacteria addition. The highest Al removal was obtained for *S. grossus* with 5% *V. alginolyticus* with an efficiency of 35.1% from 500 mg/kg initial concentration. *T. angustifolia* with 500 mg/kg initial concentration showed the highest removal of 26.2% by the addition of 5% *V. alginolyticus*. The increase of Al removal by the bioaugmentation of *V. alginolyticus* was due to the interaction in the plant's rhizosphere. Exudates of both plants provided a good environment for bacteria to live in the root area. Meanwhile, the bacteria increased the bioavailability of Al to be further extracted by plants. Certain mechanisms, such as rhizostabilisation, phyto-stimulation and phytoextraction, were considered to be the main processes that occurred during the treatment. *S. grossus* and *T. angustifolia* displayed promising ability to act as Al hyperaccumulators with bioaccumulation factor values up to 5.308 and 3.068, respectively. Development of the design of the ex-situ soil phytoremediation reactors is suggested as a future research direction because it can significantly enhance the current obtained finding.

## 1. Introduction

Aluminium (Al) is widely used in kitchen equipment, building construction material, refining industry and recycling activities. Al refining produces various types of waste, namely, skimming wastewater, ash/dross, slag and various types of impurities (Tsakiridis, 2012). The extensive use of Al has resulted in the emergence of wastes with critically dangerous effects on the environment (Supriyanto, 2009). Al inhibits the growth of plant roots (Krstic et al., 2012) and causes various diseases, such as Alzheimer's disease, amyotrophic lateral sclerosis and Parkinson's dementia, in humans (Exley, 2017; Mirza et al., 2017; Pogue and Lukiw,

2016). One example of the use of Al with harmful effects to the environment occurs in Sumobito District, Jombang Regency, East Java, Indonesia. The Al recycling industry in Sumobito District, Jombang Regency has been processing metal waste for more than 30 years (BLH Jombang, 2016). This industrial area is located close to the paddy fields and residential areas, thereby causing negative effects in the form of health and environmental disturbances (Laksono and Muzayanah, 2012). This Al recycling industry recycles slag, ash/dross and foil into economically valuable Al plates/bars. On the basis of the nature of the material category, slag, ash/dross and foil are classified as hazardous and

\* Corresponding author.

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toxic waste (HW). Accordingly, Al recycling industrial activities are classified as HW utilisation activities or businesses.

The concentrations of Al in the soil of four different locations near the industrial area of Al recycling were 35,639, 49,967, 32,916 and 23,680 mg/kg (BLH Jombang, 2016). The sampling locations were former HW dumping sites in the form of ash/dross from the Al recycling industry. The recycling industry that uses slag, ash/dross and Al foil as raw materials must implement proper waste management to reduce its negative effect that will contribute to environmental pollution and health problems. The solid waste management of the HW recycling industry is regulated under Indonesian Government Regulation No. 101 of 2014 on Hazardous Waste Management (Purwanti et al., 2018a). The residual liquid waste from this industrial activity was directly discharged into the water body without any proper treatment. Impure materials were also openly dumped in areas surrounding the industrial location. Al in environment may cause potential damages, such as toxic effects on aquatic biota, decreased soil fertility and disruption of the enzymatic activity of organisms (Krstic et al., 2012; Setiadi and Anira, 2015; Zhang and Zhou, 2005). The pollution of soil requires human intervention with the existing technology to overcome pollution (Imron et al., 2020; Menon et al., 2019). Efforts were initiated to process Al-contaminated soils by using several methods, namely, precipitation (Kurniawan et al., 2018), evaporation, ion exchange and electrolysis (Manikandan et al., 2020; Rezaee et al., 2017b, 2017a). However, some of the above-mentioned technologies have caused further issues from the chemical residue and the used energy.

Phytoremediation is a technique used to restore polluted environmental media by degrading or stabilising contaminants using plants (Lae et al., 2019; Sarwoko and Samudro, 2006). This mechanism is a sustainable and inexpensive process and a quickly developing alternative; thus, it is highly suitable for applications to remediate contaminated medium (Ghosh et al., 2006; Ismail et al., 2015). Phytoremediation features many advantages, such as environmental friendliness, easy application and low-cost maintenance, good efficiency, high aesthetic value and applicability to a variety of pollutants. Moreover, phytoremediation can be easily implemented *in situ*, addition to its low cost (Aransiola et al., 2013; Imron et al., 2019b).

In the case of Al-contaminated soil, the plants of *Scirpus grossus* and *Typha angustifolia* can be used as remediating agents. *S. grossus* is a type of plant that shows excellent potential as a heavy metal hyperaccumulator (Ismail et al., 2019; Tangahu et al., 2010). *T. angustifolia* is a grass with high resistance and plays an active and passive role in eliminating pollutants (Ahmad et al., 2017; Khan et al., 2000). Ismail et al. (2017) reported that *S. grossus* can accumulate up to 8864 mg Al/kg plant weight with an Al concentration of 100–150 mg/L. Gallon et al. (2004) also reported that the genus *Thypha* sp. had a potential in Al accumulation in their root area. Research on Al phytoremediation using Indonesian native plants is currently scarce. Bioaugmentation of bacteria to assist the phytoremediation of contaminated soil was proven to increase the pollutant removal efficiency (Ismail et al., 2019; Lebeau et al., 2011; Ojuederie and Babalola, 2017). *Vibrio alginolyticus* is a gram-negative bacteria and can remove Al up to 59.72% from 100 mg/L initial concentration (Purwanti et al., 2019b; Titah et al., 2019). However, the effect of the addition of this species to enhance the phytoremediation of Al by using *S. grossus* and *T. angustifolia* is yet to be explored. On the basis of this background, this research aims to analyse the performance of *S. grossus* and *T. angustifolia* in treating Al-contaminated soil. Moreover, this work aimed to study the effect of Al-resistant bacteria (*V. alginolyticus*) addition to the performance of Al removal. This paper covers the propagation of plants, growth rate measurement, range finding test (RFT) of contaminated soil to the used plants and bioaugmentation of *V. alginolyticus* in phytoremediation of Al-contaminated soil processes. The workflow is presented in Figure 1. The study result is expected to contribute in the field of alternative technology for Al-contaminated soil treatment.

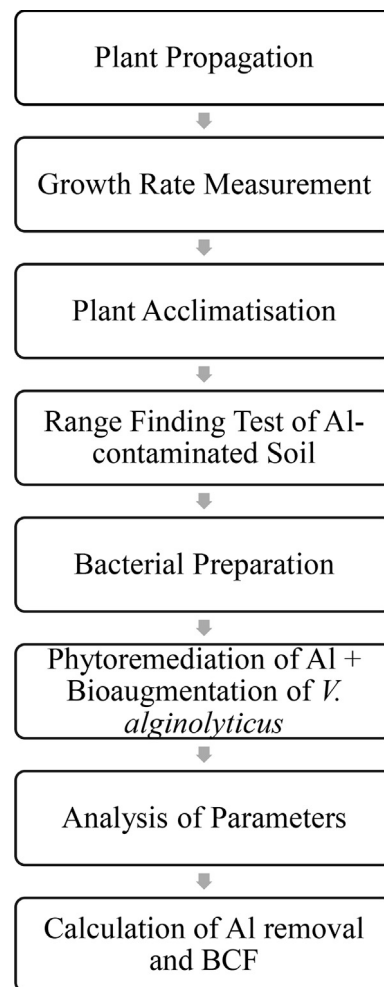


Figure 1. Research workflow.

## 2. Materials and methods

### 2.1. Source of the plants

The plants used in this study were brought from the location where *S. grossus* and *T. angustifolia* naturally thrive in large numbers. Both plants grow in habitats with soil conditions submerged in water, such as in swamps and riverbanks. *S. grossus* was obtained from the riverside area in Semut Kali, Bongkaran, Surabaya, Indonesia. Meanwhile, *T. angustifolia* was obtained from the riverside area in Rungkut Kidul, Surabaya, Indonesia.

### 2.2. Plant propagation

Plant propagation was conducted to multiply the plants needed for research. In the propagation stage, *S. grossus* and *T. angustifolia* were planted in 40 L square-shaped plastic reactors in the garden soil and routinely watered twice a day with tap water (Al concentration of 0.0098 mg/L). The characteristics of the used soil were as follows: pH 6.5, dark brown colour, 1.55 g/cm<sup>3</sup> of bulk density and 0.33 mL/g of water retention capacity. The propagation stage was performed under a greenhouse condition (temperature of 33 °C–35 °C) to obtain a second generation of *S. grossus* and *T. angustifolia* (Tong et al., 2004). This stage also aimed to determine the growth rate of *S. grossus* and *T. angustifolia*. The propagation phase was conducted for 1 month.

### 2.3. Growth rate measurement

The growth rates of *S. grossus* and *T. angustifolia* were monitored to determine the optimal plant age to be used in this study. The plant developments in terms of the length and the number of leaves were measured once every 2 days (Tangahu, 2016). Physical observation is a simple analysis. However, this mechanism is reliable when observing the growth of plants during experiments compared with other methods (Tangahu, 2016). Biochemical characterisation and pigment analyses can also be performed to observe the plant growth rate. However, such analyses are time consuming and require chemical reagents. The plant age used for the next stage was chosen by considering the generative phase to allow the maximum degradation or stabilisation of pollutants by the plants.

### 2.4. Plant acclimatisation

Plant acclimatisation aims to allow plants to adapt to the specific conditions and the medium used in the RFT and phytoremediation test (Anjum, 2015; Kozai, 1991). The acclimatisation phase was conducted for 7 days by using garden soil medium without additional pollutants and watered with tap water (Al concentration of 0.0098 mg/L). In this condition, the plants were expected to attain the characteristics of flourishing with healthy plants used in the RFT and phytoremediation test.

### 2.5. Range finding test (RFT)

The range finding test was performed to determine the ability of plants to survive at certain contaminated soil concentrations (Purwanti et al., 2018b; Tangahu et al., 2010). At this stage, *S. grossus* and *T. angustifolia* were exposed to various concentrations of Al. The reactor used was a 5 L plastic bucket containing 5 kg soil under a free-surface flow system planted with four plants. The plants used in the RFT stage were obtained from the acclimatisation stage. The concentrations of Al in soil used were 10,000, 5000, 500, 50 and 0 mg/kg (control) prepared using the spike method of  $AlCl_3$  (SAP, Indonesia) solution with soil medium. At each concentration, the plant morphology (plant height) was also observed daily. The RFT was carried out for 14 days. If the plants died or withered within this time span, then the concentration was considered high for plants to tolerate. Thus, the lethal concentrations were excluded in the next research phase (phytoremediation stage). The concentrations that can be tolerated by plants (showing no harmful effect to plants) were used in the following phytoremediation stage.

### 2.6. Phytoremediation of Al

The phytoremediation stage of the Al-polluted soil was carried out with a batch system. At this stage, 40 L plastic reactors filled with 10 kg garden soil were used. The garden soil used was dried and then sieved to obtain the uniform size. The different concentrations of Al-contaminated soil were prepared using the spike method of soil + Al solution. A 10,000 mg/L  $AlCl_3$  solution stock was mixed with soil medium under a certain ratio to obtain the desired concentration (Ismail et al., 2015). The mixture was then settled for 1 week to allow the Al to be completely spiked inside the soil matrix.

*S. grossus* and *T. angustifolia*, which had been acclimatised, were planted inside each reactor. Eight plants (single species) were used in the phytoremediation test for each reactor. The number of plants used in the phytoremediation test was calculated by upscaling the number of plants from the RFT stage. Hence, the load received by plants in the phytoremediation test was similar to that in the RFT stage. This research was conducted for 28 days with threefold replications to obtain data in triplicates. During the research, the pH was measured with a portable pH meter (OneMed, Indonesia). Approximately 1 g of soil sample was taken from the middle depth of the sand medium height (6 cm) from each reactor in the beginning and the end of the test period. Al was extracted

from soil and plant with a strong acid mixture of Aqua Regia (1:3 of  $HNO_3$  and HCl) (Hseu et al., 2002). The Al measurements were conducted with an ICP-OES (Fisher, USA) in accordance with the USEPA 6010 standard.

### 2.7. Bioaugmentation of Al-resistant bacteria

Bacterial species used in this experiment were the gram-negative bacteria of *V. alginolyticus*. Titah et al. (2019) showed that this species can remove Al from wastewater via a biosorption mechanism. The inoculum of bacteria was prepared overnight (24 h) in a nutrient broth medium (Merck, Germany), harvested with 3000 rpm centrifuge for 15 min (Macario, 2012) and diluted with physiological solution (8.5% NaCl) before application until the initial optical density of 1 Å (approximate cell counts of 9 Log CFU/ml) (Purwanti et al., 2019b). The bioaugmentation of bacteria was performed in the beginning of the phytoremediation test using v/v ratio of 2% (initial cell counts of  $6.5 \pm 0.7$  Log CFU/g) and 5% (initial cell counts of  $7.0 \pm 0.3$  Log CFU/g) (Purwanti et al., 2019c).

### 2.8. Statistical analysis

All applicable data were presented as mean  $\pm$  standard error of mean (SEM), and the statistical analysis in this research was performed using Minitab version 12.0 (Kurniawan and Imron, 2019). The analysis of correlation between the bacterial addition and the Al removal efficiency was performed using one-way analysis of variance (ANOVA) with null-hypothesis is as follows: the addition of bacteria did not significantly affect the Al removal. The alternative hypothesis is as follows: the addition of bacteria significantly affects the Al removal. Further significance analysis was conducted using Tukey HSD test. Determination of conclusion was carried out on the basis of the *p*-value with *p* < 0.05 indicating the significant difference between the results.

## 3. Results and discussion

### 3.1. Plant propagation and acclimatisation stage

Growth rate monitoring was started during sprouting of the second-generation plants. The height and number of leaves of *S. grossus* and *T. angustifolia* were measured daily (Figure 2). Meanwhile, the height and number of leaves of *S. grossus* used for the next research stage were 20 cm and 5, respectively. The height and number of leaves of *T. angustifolia* considered for next research stage were 28 cm and 5, respectively. The determination of plant height and number of leaves were carried out on the basis of the observed growth parameter after 7 days of propagation. The obtained plants then underwent acclimatisation stage. The healthy plants after a 7-day period of acclimatisation will be used in the phytoremediation test.

### 3.2. RFT of Al-contaminated soil

The Al concentration for phytoremediation test was determined through RFT. The results of 14-day observation (Figure 3) exhibited that *S. grossus* and *T. angustifolia* had symptoms of death at concentrations of 10,000 and 5000 mg/kg, respectively, after 7 days of exposure. This condition was marked by the physical changes (yellowing and withering leaves) in both plants. By contrast, the leaves, stems and roots of *S. grossus* and *T. angustifolia* turned yellow and dried on 14 days of exposure, thereby indicating that the plant cannot grow well and were about to die. The presence of these symptoms in plants was due to Al toxicity. Al causes root damage and hence inhibits the absorption of water and minerals needed by plants (Setiadi and Anira, 2015). Krstic et al. (2012) and Ismail et al. (2015) stated that the decreased growth of plants or the appearance of death symptoms are influenced by N absorption and P deficiency due to Al accumulation in plant tissues, thus decreasing the plant metabolic activity. This condition occurs because Al binds to P in

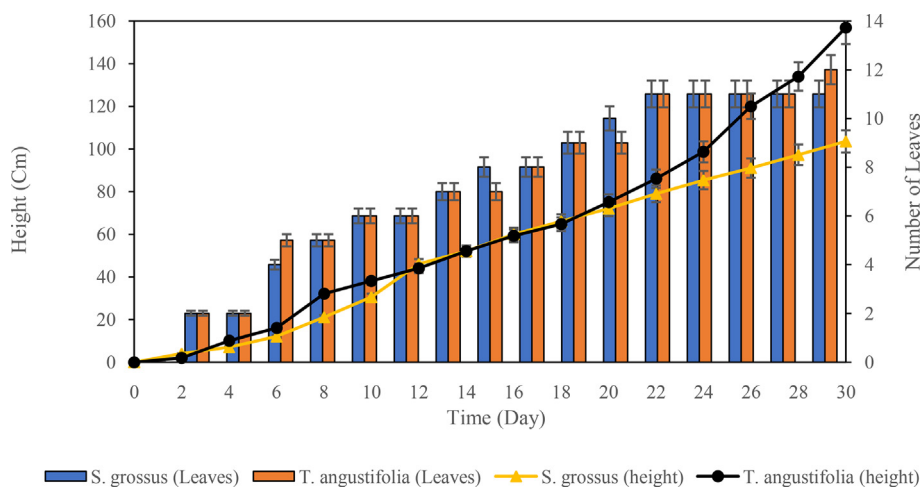


Figure 2. Initial growth measurement of *S. grossus* and *T. angustifolia*.

the form of phosphate, which is unavailable to be used by plants, thereby causing nutrient deficiency.

The plants exposed to Al solution at concentrations of 500, 50 and 0 mg/kg at the beginning to the end of the RFT stage remained fresh with no physical change. This result indicated that both plants can survive at concentrations of 0–500 mg/kg. These observations also showed that the plants can survive without being affected by the pollutant concentrations under these concentrations. The leaves of plants remained green, and the stems were still fresh. Paczkowska et al. (2007) stated that certain plants can adapt to Al because they employ a certain mechanism to suppress the adverse effects of Al to prevent it from interfering with nutrient uptake and streamline it. The Al concentration that can be tolerated by both plants in this research was 500 mg/kg. Thus, the concentration of 500 mg/kg was used for *S. grossus* and *T. angustifolia* in the phytoremediation stage.

The plant height measurements showed identical results, thereby supporting the physical observation. The height of *S. grossus* increased from day 0 to day 14 at Al concentrations of 0–500 mg/kg. At Al concentrations of 10,000 and 5000 mg/kg, the height of *S. grossus* increased on days 0–7 and then decreased on days 8–14 of exposure. Such finding was attributed to the toxicity of Al, which impeded the plant growth (Setiadi and Anira, 2015). The presence of Al causes root damage, thereby inhibiting the absorption of water and minerals required by plants (Bojórquez-Quintal et al., 2017). The height of *T. angustifolia* showed a fluctuating increase in the concentration of 0–500 mg/kg Al. Reductions in plant height in Al concentrations of 5000–10,000 mg/kg observed on the 7th day of exposure were due to Al toxicity. This observation is supported by the finding of Watanabe et al. (2001), who reported that Al poisoning affects the availability of nutrients, and the ability of plants to absorb nutrients is limited under these conditions because Al can interfere the cation exchange.

All treated reactors (T1 and T2) showed pH values ranging between 5.5 and 6.5. The pH in control reactor (T0) was gradually increased from 5.5–6.0 to 7.5. These observations indicated that precipitation by pH did not occur in a high rate for all treated reactors; however, it can still occur through complexation mechanisms (performed by root exudates or bacterial metabolisms) (Li et al., 2018; Talebi et al., 2019). On the basis of the pH observation, the removal of Al in the control reactors were carried out due to the increasing pH (Balintova and Petrilakova, 2011; Purwanti et al., 2019a), and it will be deposited in the bottom of the reactor.

### 3.3. Al content in roots and plant stems

The Al content in roots and stems was analysed to determine the amount of Al absorbed by the roots and stems of *S. grossus* and *T. angustifolia* after 28 days of phytoremediation test. This analysis was

performed at the beginning and end of the study. Figures 4 and 5 demonstrate the results of the Al test on the roots and stems of plants, respectively, in each test reactor during the observation.

The results of the initial Al content analysis of the roots and stems of *S. grossus* and *T. angustifolia* (Figures 4 and 5, respectively) show that the plants already contained Al. This finding was observed because the soil used in the propagation stage contained Al with a concentration of 1015 mg/kg. The presence of Al in the roots and stems of plants resulted from the absorption of Al from the propagation medium. The high Al content for roots was in the T1B2% reactor (*S. grossus* with 2% bacteria addition) with a value of 3265 mg/kg.

During the research, both plants healthily grow. The *S. grossus* height increased up to  $120 \pm 12$  cm, and the number of added leaves was 10. By contrast, the *T. angustifolia* height increased up to  $80 \pm 8$  cm, and the number of added leaves was 10. No sign of sclerosis or withering was observed until the end of the test period. The observed growth of plants was the main indicator that the tested species can withstand the given Al concentration and continuously perform phytoextraction (Abdullah et al., 2020; Frydenvang et al., 2015). The observed Al content for all plants was higher at the beginning of the study than that at the end due to the effect of stem growth accompanied by low Al transport from root to the stem area. Watanabe et al. (2001) reported that the accumulation of Al in stem occurred under a steady state or low rate; thus, the high growth of stem will decrease the concentration of total Al in stem (as it calculated as mg Al/kg stem). The high Al content in roots was due to their direct contact with Al-contaminated soil as the growing medium and the tendency of Al accumulation inside plants.

Al usually showed no/low accumulation in the stem and leaf parts of plants due to the tendency and the mechanism of Al accumulation inside the root area of plants (Shahandeh and Hossner, 2000). The obtained result in this study was in agreement with that of Ismail et al. (2020) who also obtained a significantly higher concentration of Al in root compared with the plant stem. Rhizostabilisation and phytoextraction played important roles in this observed result. Plant's exudates can directly stabilise/complexify the bioavailable Al in their root area (Ismail et al., 2019; Titah et al., 2018). Bioavailable Al was extracted by plants and accumulated in a certain part of plants referring to their tendency/fate (Lebeau et al., 2011). In the case of Al, the accumulation tendency is in root part.

The interaction between metals and plants facilitated by root exudates, such as amino acids, organic acids or other compounds that can accumulate metals and transport them to other plant parts, was expected to occur during this research. This finding is supported by the work of Dakora and Phillips (2002) who stated that plants that grow in soils with high Al content produce root exudates (in the form of anions of organic acids, sugars, vitamins, amino acids, purines, nucleotides and inorganic

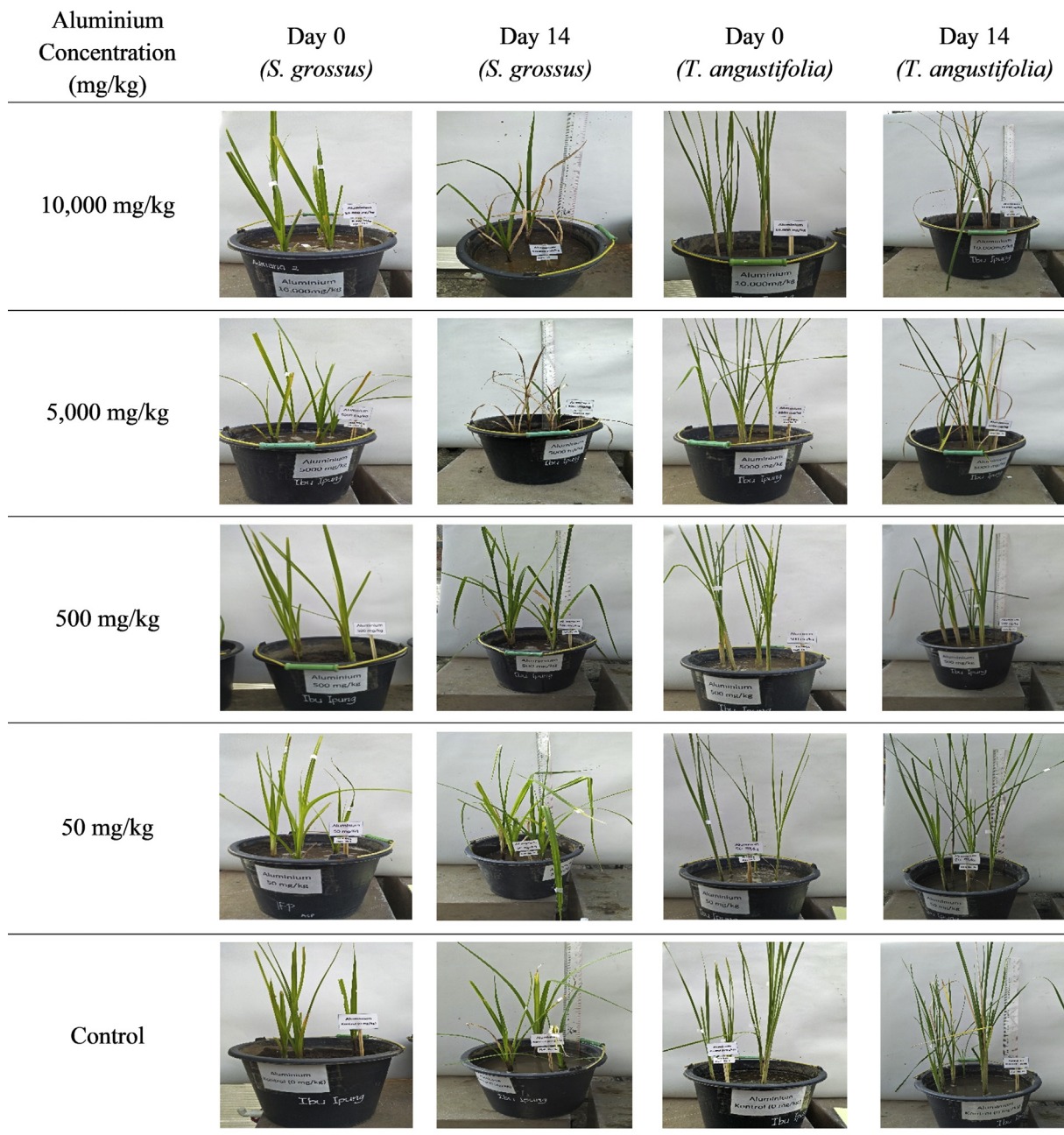


Figure 3. Observation of RFT on *S. grossus* and *T. angustifolia*.

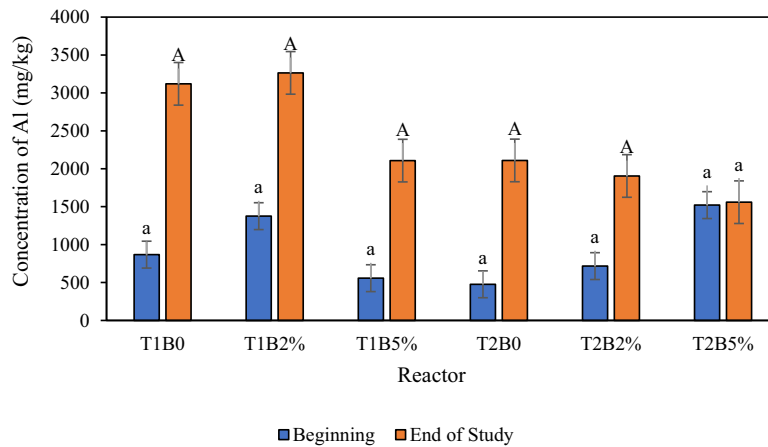
ions). These exudates help plant roots avoid the damaging effects of Al ions. Accordingly, roots can continually absorb nutrients and water. The high absorption of Al in the roots was influenced by the length during the observation period. This result is supported by the work of Sampanpanish et al. (2010) who reported that the Al accumulation increased with increasing metal concentration and time.

### 3.4. Analysis of Al content in soil

The analysis of the Al content in the soil was carried out to determine the efficiency of Al removal using *S. grossus* and *T. angustifolia* assisted by *V. alginolyticus*. The analysis was performed at the beginning and end of the study. The initial analysis was implemented to determine the initial Al content in the soil (day 0). By contrast, the final analysis was

conducted to measure the Al content in the soil after 28 days of observation. Figure 6 shows the results of the Al content in the soil in each test reactor.

Figure 6 shows that the soil in each reactor contained Al at the beginning of the study (day 0). The Al content in the soil was ranged between 786 mg/kg and 968 mg/kg. This result indicated that the soil medium used in this study already contained Al. Tsakiridis (2012) stated that Al is one of the chemical elements that constitute the earth's crust. Overall, the T1B5% reactor with *S. grossus* and the addition of 5% (v/v) bacteria during the 28 days of testing showed a low final Al content (526 mg/kg). This finding was due to the biochemical-physiological processes performed by plants and bacteria creating absorption and accumulation mechanism (Krstic et al., 2012; Ngo et al., 2014; Titah et al., 2019). The efficiency of Al removal in soil was calculated on the basis of the



Note:

T1B0 = *S. grossus* without bacterial addition

T2B0 = *T. angustifolia* without bacterial addition

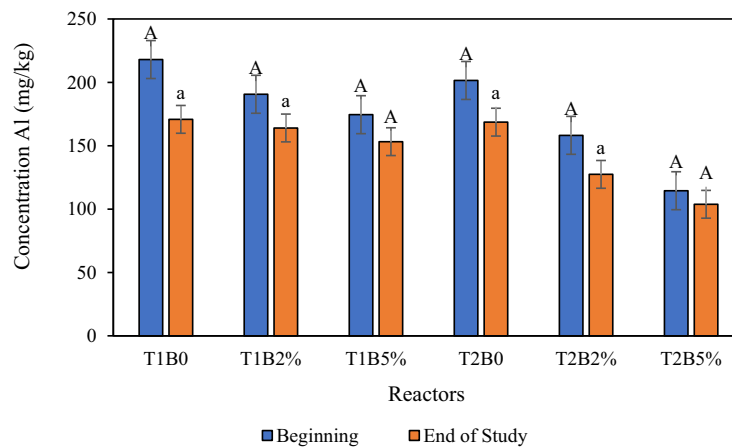
T1B2% = *S. grossus* + bacterial addition 2% (v/v)

T2B2% = *T. angustifolia* + bacterial addition 2% (v/v)

T1B5% = *S. grossus* + bacterial addition 5% (v/v)

T2B5% = *T. angustifolia* + bacterial addition 5% (v/v)

Figure 4. Al content in plant roots (values are presented in mean ± SEM). Letter a-A indicates the significant differences between the initial and the final concentrations.



Note:

T1B0 = *S. grossus* without bacterial addition

T2B0 = *T. angustifolia* without bacterial addition

T1B2% = *S. grossus* + bacterial addition 2% (v/v)

T2B2% = *T. angustifolia* + bacterial addition 2% (v/v)

T1B5% = *S. grossus* + bacterial addition 5% (v/v)

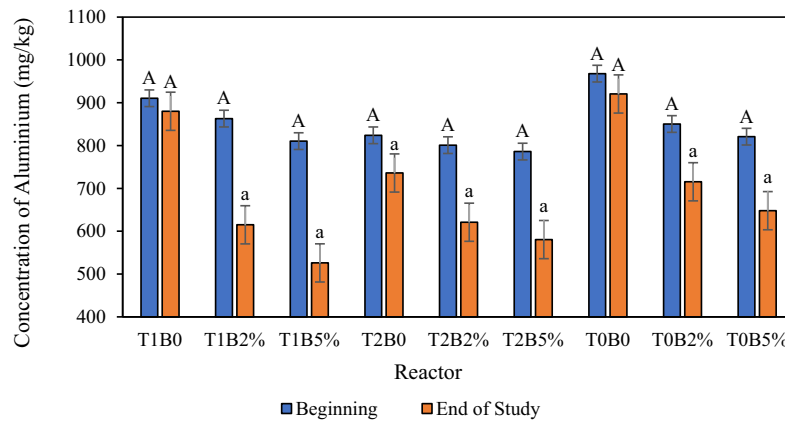
T2B5% = *T. angustifolia* + bacterial addition 5% (v/v)

Figure 5. Al content in plant stems (values are presented in mean ± SEM). Letter a-A indicates the significant differences between the initial and the final concentrations.

reduction of Al concentration in the soil medium. The efficiencies of Al removal in each reactor are shown on Figure 7.

Figure 7 shows that the high removal of Al occurred on the T1B5% reactor, which is *S. grossus*, with the addition of 5% *V. alginolyticus* reaching up to 35.1%. In comparison with T0B5% (without plants but with 5% bacteria addition), the addition of *S. grossus* significantly increased the Al removal capacity by 14.0% ( $p < 0.05$ ). *T. angustifolia* showed a lower Al removal compared with *S. grossus* with a high efficiency of 26.2% achieved with the addition of 5% *V. alginolyticus*

(T2B5%). The utilisation of *T. angustifolia* showed a significant increase of 5.1% Al removal compared with the control (T0B5%). The addition of *V. alginolyticus* showed a significant increase of Al removal compared with the no-bacterial addition reactor ( $p < 0.05$ ) with the highest increase up to 31.8% (T1B0 vs. T1B5%). This result indicated that the bacterial addition (especially pollutant-resistant/tolerant bacteria) will enhance the removal of the pollutant. Ismail et al. (2020) mentioned that the addition of bacteria promotes the growth



Note:

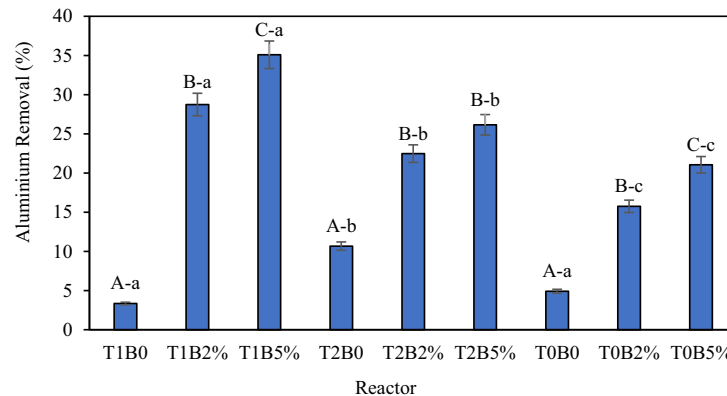
- T1B0 = *S. grossus* without bacterial addition
- T1B2% = *S. grossus* + bacterial addition 2% (v/v)
- T1B5% = *S. grossus* + bacterial addition 5% (v/v)
- T2B0 = *T. angustifolia* without bacterial addition
- T2B2% = *T. angustifolia* + bacterial addition 2% (v/v)
- T2B5% = *T. angustifolia* + bacterial addition 5% (v/v)
- T0B0 = Without plant and bacteria
- T0B2% = Without plant + bacterial addition 2% (v/v)
- T0B5% = Without plant + bacterial addition 5% (v/v)

Figure 6. Al content in the soil (values are presented in mean ± SEM). Letter a-A indicates the significant differences between the initial and the final concentrations.

of plants and enhance the Al removal by up to 19%. The proposed mass balances of Al for control and treated reactors are presented on Figure 8.

The addition of bacteria might assist the phytoremediation through some mechanisms, including phytostimulation and rhizostabilisation (Ebrahimipour et al., 2020; Ismail et al., 2019; Tangahu et al., 2011).

Plant's exudates create a good environment for bacteria to live in the root area exhibiting the phytostimulation mechanism. Exudates also provide some essential elements that bacteria need to perform enzymatic reactions (Dakora and Phillips, 2002). Bacteria assist the removal of pollutant by transforming the pollutant into a less toxic compound,



Note:

- T1B0 = *S. grossus* without bacterial addition
- T1B2% = *S. grossus* + bacterial addition 2% (v/v)
- T1B5% = *S. grossus* + bacterial addition 5% (v/v)
- T2B0 = *T. angustifolia* without bacterial addition
- T2B2% = *T. angustifolia* + bacterial addition 2% (v/v)
- T2B5% = *T. angustifolia* + bacterial addition 5% (v/v)
- T0B0 = Without plant and bacteria
- T0B2% = Without plant + bacterial addition 2% (v/v)
- T0B5% = Without plant + bacterial addition 5% (v/v)

Figure 7. Efficiency of Al removal (values are presented in mean ± SEM). Letter A-B-C above the graph indicates the significant difference of Al removal from soil medium between no bacterial addition, 2% bacterial addition and 5% bacterial addition; letter a-b-c above the graph indicates the significant difference of Al removal from the soil medium between *S. grossus*, *T. angustifolia* and control reactor.

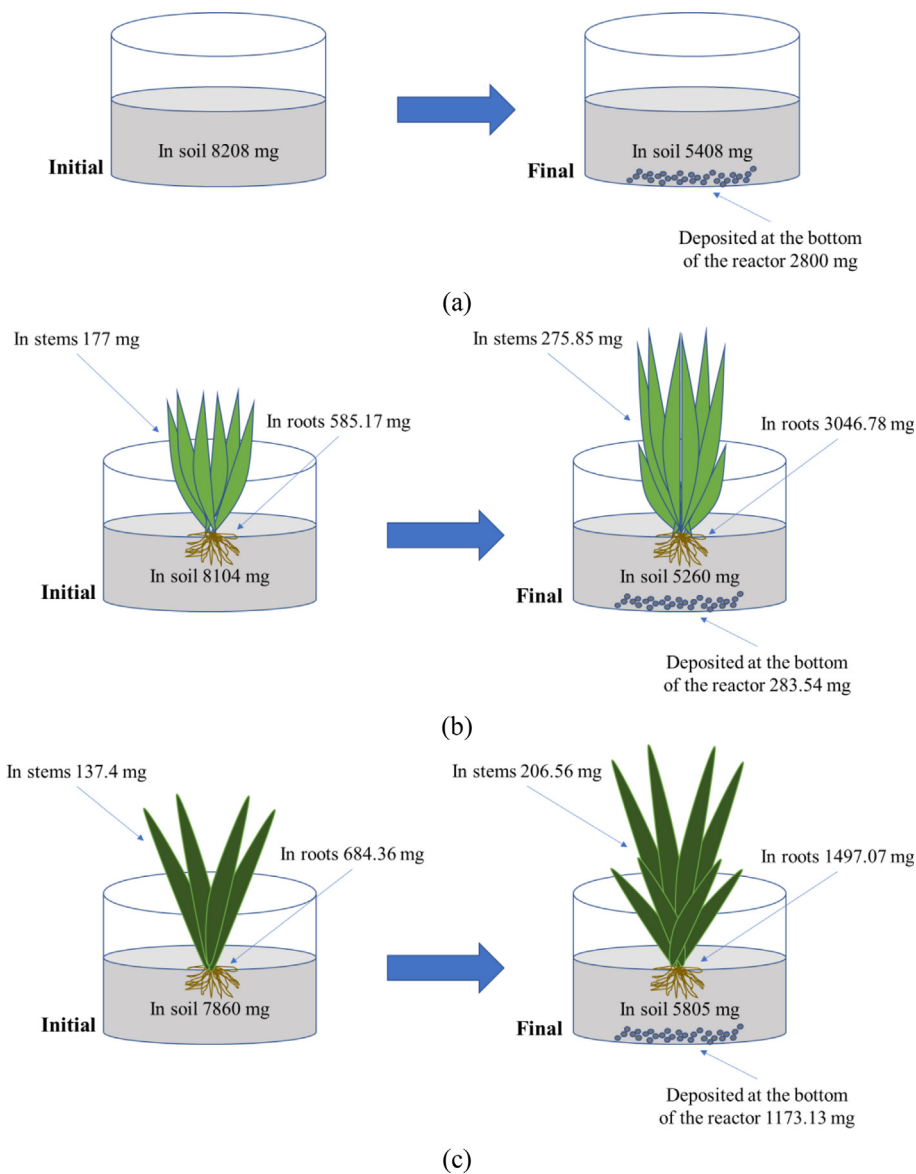


Figure 8. Proposed mass balance for (a) T0B5% (b) T1B5% (c) T2B5%.

which can then be extracted by plants (Mohan and Tippa, 2019). Moreover, bacteria can increase the bioavailability of pollutant (especially metal); thus, phytoextraction might occur in a high rate (Imron et al., 2020). Understanding how the Al removal occurred during the study, it is strongly suggested that the *in-situ* application of this method must be accompanied with underlayer/liner protection to prevent the infiltration of metal ion that might pollute the groundwater further (Imron et al., 2019a). Layer need to be placed below the area of the remediated soil (by excavating) to limit/block the percolation of water that carries soluble metal ion (Adar and Bilgili, 2015). Several layer types can be used on the application, including geomembrane and clay (Abd-Elhamid et al., 2019).

No standard for maximum/allowable concentration of Al in soil is available at present. Several studies also reported a high concentration of Al in soil because it is an abundant metal element in the earth's crust. Rasmussen et al. (2001) reported a concentration higher than 12,000 mg/kg of Al on the 160 sampling sites in the province of Canada. Zolotajkin et al. (2011) also reported the concentration of Al up to 4800 mg/kg in some sampling locations in Poland. The toxicity of Al depends on the pH condition. A concentration starting from 300 mg/kg in acidic condition of soil might cause toxicity to plants (Zolotajkin et al., 2011),

thereby resulting in a withering of leaves by interfering the essential elemental uptake (Ismail et al., 2020). Sequential/multiple stages of phytoremediation can be performed (Said et al., 2020) by using the tested plants in this study to achieve the concentration below 300 mg/kg.

### 3.5. Bioaccumulation of Al by plants

The assessment of bioconcentration factor (BCF) aimed to determine the ability of plants to accumulate Al from the contaminated medium. BCF was calculated on the basis of the ratio of the metal concentration in plant parts with the metal concentration in the growing medium (Santiago et al., 2011). The BCF value more than unity indicated the capability of plants in performing hyperaccumulation of metal (Baker and Brooks, 1989; Bolan et al., 2011). The BCF was calculated for the final concentration of Al in plants (roots and stems). The soil medium is presented on Table 1.

Table 1 demonstrates that *S. grossus* and *T. angustifolia* can be categorised as hyperaccumulator plants. In accordance with the obtained result, Tangahu et al. (2010) also stated that *S. grossus* exhibits its capability as a hyperaccumulator plant on the basis of the bioaccumulation of pollutants. Tahira (2006) characterised *T. angustifolia* as



**Table 1.** BCF of Al by *S. grossus* and *T. angustifolia*.

Reactor	Aluminium Concentration in Medium (mg/kg)	Aluminium Concentration in Plants (mg/kg)		BCF Value
		Root	Stem	
T1B0	880.1	3,120	218	3.545
T1B2%	615	3,265	190.6	5.308
T1B5%	526	2,108	174.5	4.007
T2B0	736	2,110	201.5	2.866
T2B2%	621	1,905	158.2	3.068
T2B5%	580.5	1,559	114.5	2.686

a hyperaccumulator plant due to its high ratio of metal bioconcentrations in roots and other plant parts. All *S. grossus* reactors showed higher BCF values compared with all *T. angustifolia* reactors. This situation demonstrated that *S. grossus* has a good performance in the bioaccumulation of Al from the contaminated medium.

#### 4. Conclusions

The RFT result showed that *S. grossus* and *Thya angustifolia* can tolerate Al in soil up to 500 mg/kg. The addition of *V. alginolyticus* showed a significant increment in the Al removal from soil reaching up to 14.0% in the *S. grossus* reactor with 5% bacterial addition compared with the no-bacteria addition (control). The maximum Al removal was obtained in a reactor of *S. grossus* plant with 5% bacteria addition with the removal efficiency of 35.1%. Meanwhile, a removal efficiency of 26.2% was observed for *T. angustifolia* with 5% bacteria addition. Bio-augmentation of *V. alginolyticus* enhanced the overall Al removal due to the improvement of Al bioavailability, which can be further extracted from soil by plants. Rhizostabilisation, phytostimulation and phytoextraction mechanisms were subjected to be occurred during the treatment. Both plants have shown potential as Al hyperaccumulators with BCF values up to 5.308 and 3.068 for *S. grossus* and *T. angustifolia*, respectively. The presented result was limited to the indirect analysis of the enhancing effect of Al removal by bacteria addition; thus, a further study on the interaction of plants and bacteria in phytoremediation of Al is highly suggested. A further study on the design of the reactor for a real *ex situ* treatment application can be carried out to enrich the current findings. A direct *in situ* application can also be applied by planting the above-mentioned plant species and introducing the *V. alginolyticus* bacteria into the soil under several parameter control to maintain the optimal growth of plants and bacteria.

#### Declarations

##### Author contribution statement

Ipung Fitri Purwanti, Siti Rozaimah Sheikh Abdullah: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Adriana Obenu, Setyo Budi Kurniawan: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Bieby Voijsant Tangahu: Conceived and designed the experiments.

Muhammad Fauzul Imron: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

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##### Competing interest statement

The authors declare no conflict of interest.

##### Additional information

No additional information is available for this paper.

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