

UNLOCKING THE POTENTIAL OF ELECTROKINETIC (EK)-ASSISTED PHYTOREMEDIATION ON ACIDIC SLOPE SOIL

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Abstract. The combination of phytoremediation and electrokinetic remediation is an attempt to accelerate the process of soil rehabilitation and overcome the limitations of phytoremediation. This current study was undertaken to assess the influence of electrokinetic-assisted phytoremediation on plant growth and soil quality under acidic conditions. A total of 40 potential slope plants, namely *Melastoma malabathricum* and *Syzygium campanulatum* were grown in 60 pots in the following six treatments; soil (as control – T1); soil + EK (T2); *M. malabathricum* (T3); *M. malabathricum* + EK (T4); *S. campanulatum* (T5); and *S. campanulatum* + EK (T6). After twelve weeks of observation, the EK-assisted treatments (T4 and T6) exhibited increment in soil pH by 4.0% and 5.3%, respectively. The plant growth performance of both species was observed to decline under EK treatments. Despite the lower biomass, the plants treated with EK approach contain higher concentrations of iron (Fe) and aluminum (Al). These plants are also able to improve the soil pH as well as conductivity level in soil, thus, greatly alleviating the soil acidity problem. Further *ex situ* investigation is essential to truly establish the practicality of utilizing both species as phytoremediator with the assistance of electrokinetic remediation.

Keywords: soil acidity, bioavailability, *M. malabathricum*, *S. campanulatum*, combined remediation techniques

Introduction

Addressing the problem of acidity is one of the topical issues of sloping lands. Generally, slope soil is acidic can be attributed to the naturally prolonged and pervasive weathering of rocks, or developed as the result of anthropogenic activities. Graphitic schist, is one of the common parent materials in Peninsular Malaysia, and the residual soils derived from this rock type is commonly acidic and landslide prone (Kong and Rahman, 2000; Regmi et al., 2016). Moreover, a study by Kong (2017) reported that the cut slope failures frequently occurred in the graphitic schist derived materials, that frequently involving acid sulphate soils and pyrite minerals. The exposure to the warm and wet climates of this country would result in excessive pyrite oxidation and hydrolysis reactions, as part of chemical weathering processes, which will produce sulphuric acid (H₂SO₄) and iron oxides (Fe(OH)₃), consequently, contributing to extreme acidic condition of the soil. This may further cause the release of soluble aluminium (Al) due to dissolution of available metals in soil, disintegration of the soil structure, reducing soil stability and eventually, the slope failures (Kong and Rahman, 2000; Sparks, 2003; Nkpadobi et al., 2014). Furthermore, this acidification will also turn the slopes into barren and infertile due to accumulation of metals in soil, hence, ensues lower plant bioavailability and lower plant coverage (Harter, 2007; Bakhshipour et al., 2016; Dislich

et al., 2017). Subsequently, a reduce plant coverage leads to decrease of biogeochemical cycling restoration, and eventually it expedites soil erosion and degradation (Bowyer, 2006; Dislich et al., 2017). Therefore, a concerted effort to alleviate, remediate and rehabilitate the acidic condition of the slope is indeed essential.

Amongst the rehabilitation strategies, a bioengineering technique using vegetation as protection materials on the slope is regarded as one of the most promising techniques for slope management (Osman and Barakbah, 2011; Bella et al., 2017; Boldrin et al., 2017). In addition, phytoremediation technique is one of the good treatments available in bioengineering to restore the highly acidic slope condition (Patel, 2019). Phytoremediation refers to the inherent ability of certain plant species to accumulate and render harmless the toxic or excessive metals in soils into their vegetative or reproductive parts (Charlesworth et al., 2020; Manoj et al., 2020). Phytoremediation strategies can be classified into four, which are phytostabilization, phytoextraction, phytovolatilization and phytofiltration (Merkel and Hoyer, 2012; DalCorso et al., 2019) and these processes could be varied between the plant species. In general, phytostabilization reduces metal bioavailability in soil, phytoextraction uptakes metals from the soil into the plants and translocate in their aboveground biomass, phytovolatilization releases toxic elements from soil into the atmosphere through plants in harmless volatile form, and phytofiltration absorbs the metals from the hydro-environment through the plant parts (Marques et al., 2009; Jacob et al., 2018; Yan et al., 2020).

Nevertheless, this straightforward approach of phytoremediation is time-consuming with low-efficiency due to the slow plant growth (Ali et al., 2013). Thus, in this study, we proposed combination of electrokinetic-assistance to overcome this limitation. Electrokinetic(EK) remediation approach is an environmental technique that is used to remove contaminants in soil without excavating the soil for the purpose of decontamination (De Battisti and Ferro, 2007; Reddy and Cameselle, 2009). The provision of low-intensity electric potential by the EK process could enhance the bioavailability of excessive metals for desorption and transportation, as well as promoting the plant growth and reducing the period of phytoremediation (Bedmar et al., 2009). The migration of contaminants from the deep soil to plant roots is executed by electromigration and electroosmosis mechanism of the EK (Bedmar et al., 2009). Lower voltage gradient (4 V/cm) applies to the contaminated soil for 8 hours is beneficial to ensure better accumulation of heavymetals/ nutrients near the electrodes area (Kornilovich et al., 2005).

Additionally, selection of suitable remediating species (hyper-accumulators) is also a key, with promising characteristics such as the high tolerance to acidity, fast growing with high biomass, extensive root system, and less nutrient requirement to grow (Manoj et al., 2020), whereby using unsuitable species may result in no changes or adverse effects on the slope soil. More than 450 plant species have been identified as metal hyper-accumulators (Suman et al., 2018), but limited species are suitable for slope treatment plants. Study by Watanabe and Osaki (2001) discovered that *Melastoma malabathricum* was known as the hyper-accumulator plant that usually grows in tropical acid soils. This species can grow well in poor soil conditions; low nutrients, high concentration of aluminium (Al), and low pH level (Osman et al., 2014). Whilst *Syzygium campanulatum* is usually found in the urban and coastal areas and known as a fast-growing plant, which able to grow independently (Roseli et al., 2010). This plant also known for its ability to absorb contaminants from soil such as ammonium nitrate and has higher chances of

adaptability towards new environment (Ling et al., 2009; Arunbabu et al., 2015). Hence, the above two slope species have been chosen for their potential acidic tolerant plants.

The main objective for this study is to evaluate the effects of electrokinetic-assisted approach to phytoremediation performance and soil acidity improvement, by considering the plant biomass and root growth. Moreover, the concentration of selected metals in the plant tissues, has been determined and the bio-concentration factor (BCF) and the translocation factor (TF) were also assessed.

Materials and methods

Plant material and experimental design

Pot experiment was performed in a glasshouse at Rimba Ilmu, Universiti Malaya Kuala Lumpur, Malaysia (3°7'52.1076"N, 101°39'25.218"E). The glasshouse received a range of Photosynthetically Active Radiation (PAR) between 300-2000 $\mu\text{E mol m}^{-2} \text{s}^{-1}$, relative humidity (RH) and atmospheric temperature of 65-90% and 25-28 °C, respectively.

A total of 60 pots (27 cm $\times$ 20 cm $\times$ 17 cm) were filled with silty clay soil with total soil volume of 7803 cm³. The soil was collected from the cut slope area located at the Bukit Beruntung, Rawang, Selangor. There were six treatments in this study: soil only (control – T1); soil + EK (T2); *M. malabathricum* alone (T3); *M. malabathricum* + EK (T4); *S. campanulatum* alone (T5); and *S. campanulatum* + EK (T6). Each treatment was carried out with ten replicates and monitored for twelve weeks.

Plant materials

The species studied; *M. malabathricum* and *S. campanulatum* were propagated in polybags before being transplanted to the experimental pots. The twenty seedlings of each species with uniform height of 30 cm were selected and transferred into the pots (27 cm $\times$ 20 cm $\times$ 17 cm) for further investigation. The pots were arranged in a complete randomized design (CRD).

Electrokinetic (EK)-assisted phytoremediation

A pair of copper electrodes were used and were vertically inserted into the soil with 8 cm spacing between each other to allow electroosmotic fluxes induced by the applied electric field in the soil. Plant species was positioned at the middle of the electrodes, where each electrode was 4 cm apart from the plant stem (*Figure 1*). A low intensity direct current (DC) of 4V was applied for 8 hours on/ 16 hours off per day (Cang et al., 2011) for 12 weeks. Each pot was irrigated daily (600 mL) to maintain the soil water content to be at 60% of water holding capacity, and the plants did not receive any fertilization during the experiment.

Parameters measurement

Soil pH and electrical conductivity (EC)

Both the soil pH and electrical conductivity (EC) were measured during initial and end of the experiment. The 10 grams (g) of soil samples were air-dried at room temperature until constant weight and were sieved through a 2-mm mesh sieve. Soil solution was made up of 1:2.5 of soil to ultra-pure water (UPW) and soil pH was determined by using a pH

meter (PB-10 Sartorius GmbH, Gottingen, Germany). By using the same samples, the volume of 25 mL of UPW was doubled and the samples were then shaken mechanically at 15 rpm for an hour to dissolve the soluble salts. The EC value was recorded using a conductivity benchtop meter with automatic temperature compensation (HI-2315, Hanna Ins., US). Altogether five pots from each treatment were sampled and both parameters were measured in triplicate.



Figure 1. The vertical insertions of electrodes (yellow arrows) into the soil. Plant species (*S. campanulatum*) was positioned in between the electrodes

Plant height, total biomass and root length

Plant height of both species with five replications for each was measured at initial and end of the experiment by using a measuring tape. Plant biomass was recorded at harvest. All individual plant roots were gently washed with tap water to remove soils and then were partitioned into roots and shoots. Samples then were oven-dried at 80 °C until the constant weight and the dry mass was recorded by using the electronic balance. Five samples of cleaned roots were positioned in a scanner and based on the scanned images, the total root length were determined by using the WinRHIZO software (Pro program, Regent Instruments Inc., Canada) in triplicates.

Soil particle size distribution

Dry soil sample was weighed for 500 g. The weight of the sieves and the pan that would be used during the analysis were also recorded. Each sieves were thoroughly cleaned up before the test. The sieves were assembled in ascending order, in which the larger openings sieve was on top; the No. 4 (4.75 mm) on top and the No. 200 (75 µm) sieve on the bottom of the stack. The soil sample was placed into the top sieve and a cover/ lid was placed over the sieve. The sieves stack was placed on the mechanical shaker and was shaken for 10 minutes. The weight of each sieve and its pan were recorded. The weight of the soil retained on each sieve was calculated to obtained the percentage of particle size distribution of the soil (Choate et al., 2006; Geoengineer, 2021).

Metal concentrations in soil and plant samples

Iron (Fe) and Al concentrations were determined by using an inductively coupled plasma optical emission spectroscopy (ICP-OES) (Optima 7300 DV, Perkin Elmer, USA). Five samples with the mass of 1 g of each soil and plant part was digested beforehand using microwave (The Titan MPS, Perkin Elmer, USA) following sample preparation system with aqua regia mixture of 65% (v/v) nitric acid (HNO₃) and 37% (v/v) hydrochloric acid (HCl). Duplicate blank and laboratory standards were analysed routinely for quality assurance purposes.

Bio-concentration (BCF) and translocation factors (TF)

The bio-concentration factor (BCF) was calculated as the ratio of metal concentration in the plant at harvest to the concentration of the metal in soil medium (Zhuang et al., 2007) (Eq. 1). Whilst the translocation factor (TF) (Eq. 2) is defined as the ability of plant to translocate the metals the root to the shoot systems (Padmavathiamma and Li, 2007).

$$BCF = \frac{\text{Metal concentration in the plant (roots, stems, and leaves)}}{\text{Metal concentration in the soil}} \quad (\text{Eq.1})$$

$$TF = \frac{\text{Metal concentration in shoot (stems and leaves)}}{\text{Metal concentration in root}} \quad (\text{Eq.2})$$

Statistical analysis

A one-way analysis of variance (ANOVA) were performed for comparing means between treatments of twelve replications (n=12). Differences between means were compared and a post-hoc analysis of variance was determined using the Tukey's method at 0.05 probability level. Whilst the t-test was analysed for comparing means of initial and end of experiment. These statistical analyses were performed using the SPSS 20.0 statistical package (SPSS Inc, Chicago, IL, USA).

Results and discussion

Physical and chemical characteristics of soil

The study area is underlain by graphitic schist rock type. The surface exposure of graphitic schist to the repetitive wet and dry conditions has accelerated the process of weathering, which contributes to severe soil acidification and the unusual concentrations of metals in the slope soil. Furthermore, the very low soil pH level has resulted in generating acid drainage, as well as the degradation of slope construction materials such as drainage and ditches. Physical and chemical characteristics of the collected soil were presented in *Table 1*.

Fe and Al concentration in soils

In the collected soil (*Table 1*), the anion was dominated by the sulphate (SO₄) whilst for the cations, the iron (Fe) and aluminium (Al) are abundant in the soil. The Fe and Al were further assessed as the target metals due to the fact that vegetation absorbs more positively charged ions (cations) compared to the anions hence, these toxic metals are more closely allied to the topic of phytoremediation.

Table 1. Physical and chemical characteristics of the sample soil

PARAMETER	VALUE / RANGE
pH	3 - 4
Sand (%) (w/w)	16.4
Silt (%) (w/w)	40.4
Clay (%) (w/w)	43.2
Electrical Conductivity (mS cm ⁻¹)	0.363±0.07
Total SO ₄ (mg/kg)	12,587.1±2,555.67
Total iron (Fe) (mg/kg)	23,756.67±3,373.96
Total aluminium (Al) (mg/kg)	20,238.33±882.56

Soil pH and electrical conductivity (EC) changes

After the twelve-week of experiment, T3 recorded significantly highest increment of soil pH value which is by 6.2% whilst the lowest was in T4. Treatments with EK-assisted phytoremediation (T4 and T6) showed the pattern of increment in soil pH (*Figure 2*). These findings demonstrated the good perspectives of the EK assistance in phytoremediation by facilitating the mobility and bioavailability of the contaminants for plants uptake (Cameselle et al., 2013). In addition, increased of pH in acidic soil is also associated with a remarkable decrease in metals concentration in soils (McKee Jr and McKevlin, 1993; George et al., 2012), indicating the ability of the species studied to accumulate the excessive metals in soils. In overall, *S. campanulatum* treated with EK showed a significant increment in soil pH value compared to a slight increment showed by *M. malabathricum* treated with EK.

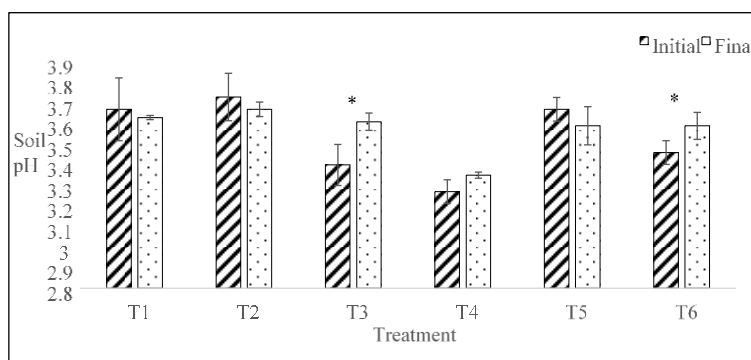


Figure 2. The soil pH value during initial and end of experiment. Vertical bar represents standard deviation. * denotes significant difference at $p \leq 0.05$, ($n=12$)

All treatments presented an increased electrical conductivity (EC) value at the end of experiment with the highest increment was shown in T1 and the lowest in T5 by 177.7% and 34.4%, respectively (*Figure 3*). The higher increment of EC in EK-assisted treatments (T4 and T6) implies that direct current (DC) used in EK had increased the production of metal ions at cathode and anode regions due to electroosmotic flow, thus, increase the conductivity in soil (Chang et al., 2019). In other way, highest conductivity in control (T1) was due to the presence of high metals concentration in soils, contributing to increase the soil acidity (Attah and Regasa, 2013).

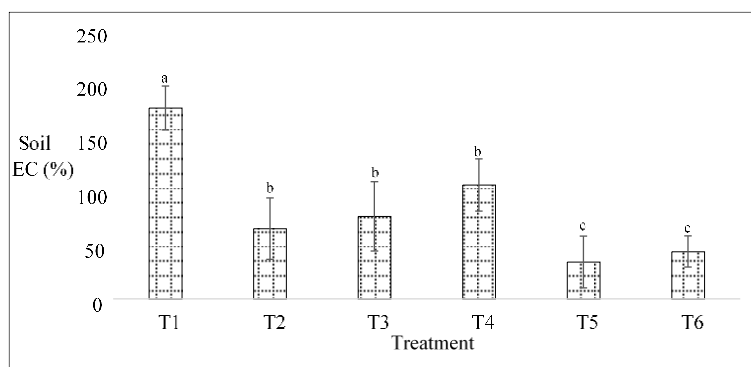


Figure 3. Percentage of changes (increment) in soil EC among treatments. Different letters showed significant difference at $p \leq 0.05$, ($n=12$)

Plant height, total biomass and root length

As can be seen in Figure 4a-c, the value of total plant biomass, root length, and plant height for both species were significantly higher in non-EK treatments (T5 and T3). The value of dry weight biomass of *M. malabathricum* in T4 was trifold lower than those in T3, whilst the *S. campanulatum* in T6 was 52.4% lower than those in T5. One of the possible reasons for getting poor growth of both plants was due to the greater phytotoxicity of metals on plants (O'Connor et al., 2003). Although previous studies allegedly proved that both species are tolerant to highly acidic soils (Watanabe and Osaki, 2001; Osman et al., 2014; Arunbabu et al., 2015), the increased of metal bioavailability in soil by the electric field might provoked higher uptake by plants (Chibuike and Obiora, 2014), thus, interfere the metabolic processes of the plant species.

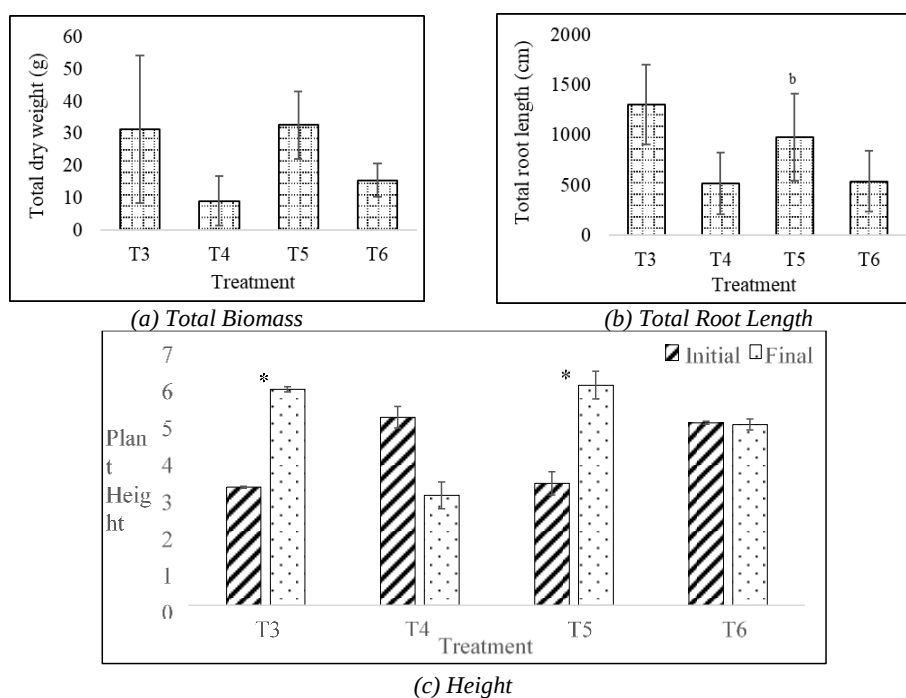


Figure 4. (a) The Total Biomass, (b) Total Root Length, and (c) Plant Height of the species studied in each treatment. Vertical bar represents standard deviation. Different letters and * denotes significant difference at $p \leq 0.05$, ($n=12$)

In addition, the applied voltage of 4V in both T4 and T6 exerted a negative effect on total root length of both species studied. Findings by Cang et al. (2011) in Indian mustard species also reported a decline in root biomass in 4V treatment while the bioavailability of heavy metals was enhanced on the similar voltage treatment. We infer that there was a trade-off between the metal bioavailability and the adverse effects of the EK on the plant growth development. Therefore, both plant species are postulated to be EK-sensitive thus, reduced the total root length. Whilst in terms of the plant height, *S. campanulatum* in T6 was seemingly not affected by the EK application, suggesting the independent behaviour of each species against the electric field.

Iron (Fe) and aluminium (Al) in plant

For iron (Fe) concentration in plant parts, the highest concentration value was recorded in roots of *M. malabathricum* in T4 (Figure 5a). Similarly, the highest concentration for leaves and stems were also recorded in T4 with 425.23 and 169.53 mg/kg, respectively. For *S. campanulatum*, there was no significant difference was found between the T5 and T6 in all plant parts. Meanwhile, the concentration of aluminium (Al) in all parts (except the plant leaves) of both species in all treatments were similar, either with or without EK treatments (Figure 5b). These results imply that *M. malabathricum* and *S. campanulatum* can accumulate Al regardless their poor plant growth performance, showing their capability as Al accumulator plants.

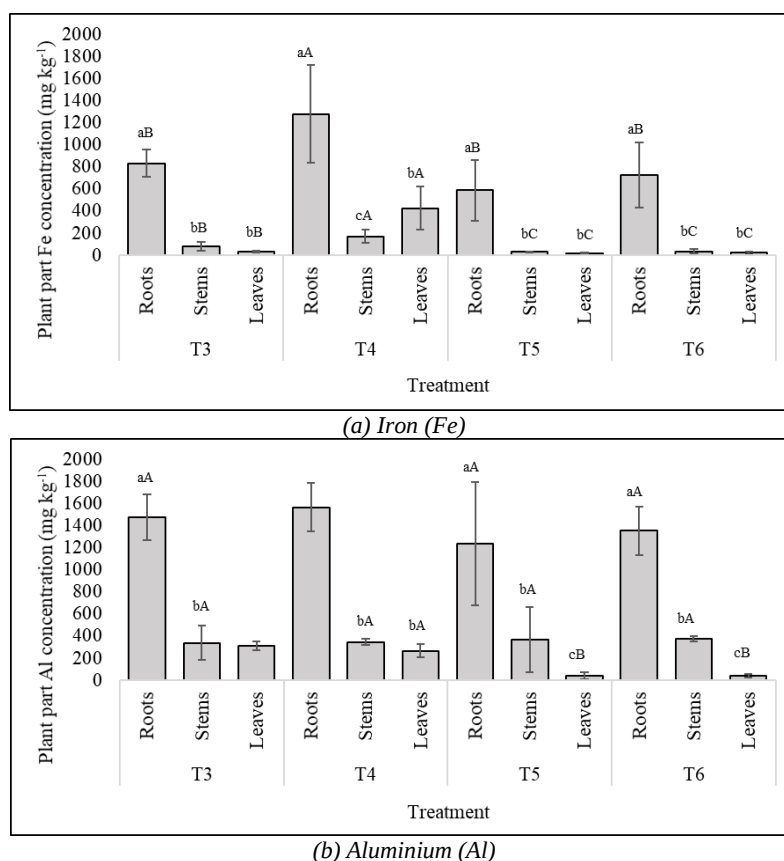


Figure 5. Concentration of (a) iron (Fe) and (b) aluminium (Al) in plant parts of each treatment. Vertical bar represents standard deviation. Different lowercase letters indicate significant differences between plant parts for each treatment, and different capital letters indicate a significant difference between treatments for each plant part ($p < 0.05$), ($n=12$)

By comparing the Fe and Al distribution in different plant parts, we can observe that the concentrations of both metals were significantly higher in roots than those in stems and leaves, suggesting that longer cultivation times are necessary for the plants to transport the metals from the roots to the upper parts (Cameselle et al., 2013). Interestingly, despite exhibiting the lowest in total dry weight biomass, *M. malabathricum* (T4) accumulated the greatest amount of metals concentration in its plant organs. Based on this finding, *M. malabathricum* as Al accumulator with high metals accumulation in small volume of biomass, it is recommended that this plant able to be grown in acidic soil condition as it is tolerance to such stressful condition and able to concentrate high level of both essential and toxic elements in their foliage (Mao et al., 2015).

The application of electric field has created the acid front at the anode and base front at the cathode, generating the mass transfer of charged ions towards the electrode regions and allows the ions uptake by the plant roots at the inter-electrode region (FRTR, 2020). Furthermore, the increase of ion mobility in soil would contribute to the charge of the surface becomes less positive, hence, providing less negative sorption plane for the anions in soil (Chad and James, 2019). This latter will transform the metals into a bioavailable form (Roy et al., 2013), thus, improve the acidic condition of the soil.

Bio-concentration (BCF), and translocation factors (TF)

The metal concentrations determined in soil and plant were used to calculate BCF and TF to determine plant phytoremediation potential (Usman et al., 2019). In this study, the calculated bio-concentration factor (BCF) for both Fe and Al demonstrated that *M. malabathricum* in T4 had higher BCF values compared to other treatments (Table 2). Both species displayed the BCF values less than 1, in which the species that having BCF more than 1 (≥ 1) is considered to be ideal hyper-accumulator (Sinha et al., 2007). On a similar note, the translocation factor (TF) values of both metals in all treatments were also less than unity, indicating the plant remediates the metals by phyto-stabilization through concentrating the metals in the root rather than in shoot which suggested ineffective metal transfer (Yoon et al., 2006).

Table 2. Bio-concentration factor (BCF) and translocation factors (TF) of Fe and Al of each treatment. Different lowercase letters indicate a significant difference between treatments ($p < 0.05$)

Treatment	Bio-concentration factor (BCF)		Translocation factor (TF)	
	Fe	Al	Fe	Al
T3	0.04±0.013 ^b	0.105±0.048 ^a	0.131±0.061 ^c	0.439±0.18 ^c
T4	0.079±0.032 ^a	0.107±0.062 ^a	0.466±0.2 ^d	0.389±0.114 ^c
T5	0.029±0.009 ^b	0.081±0.026 ^b	0.07±0.033 ^a	0.266±0.097 ^b
T6	0.033±0.016 ^b	0.087±0.037 ^b	0.04±0.019 ^a	0.032±0.01 ^a

Nevertheless, the total amount of >1000 mg/kg of Fe and Al accumulated in both plants (as shown in Figure 5) could help to describe the tolerance mechanisms and the ability of both species to accumulate the metals. Additionally, the application of EK field helps in increasing metal bioavailability in the soil, hence, increased the rate of metals uptake by the root, the bioaccumulation as well as the translocation in the plant (Nirola et al., 2015).

EK assisted phytoremediation and plant adaptability

Phytoremediation rises as a promising environmental friendly technology that can mitigate the acidity problem of the soil. Over the past decade, the electro-kinetic remediation coupled with phytoremediation has been proposed as a feasible alternative to overcome the limitations of phytoremediation such as lower plant bioavailability and long treatment time (Couto et al., 2015; Hassan et al., 2018). In this pot experiment, overall results showed that within the period of twelve weeks, this combined mechanism of EK-phytoremediation reveals remarkable findings in alleviating the acidity and enhance the remediation process of the studied soils.

In terms of soil acidity improvement, the application of EK had a significant positive effect on the pH value increment, may be due to the greater metals uptake in rhizospheric region (Nair et al., 2007). The electric field by EK had generated the electromigration and electrolysis activities in soils, which favour the mobility and bioavailability of the metals. These latter would enhance the phytoextraction by plants, and eventually, improve soil pH and alleviate the soil acidity (Selvi et al., 2019; Voccianti et al., 2021). Besides, the increment of soil EC value was also found greater with EK-assistance (T4 and T6) compared to plant species alone, indicating the combination of the ions in root exudates and electric currents is beneficial to increase the EC, thus, could enhance the transportable metals for removal and nutrients for the plant growth mechanism (Sánchez et al., 2020).

Additionally, we observed that the species studied, both *M. malabathricum* and *S. campanulatum* survived under the concentrated acidic condition (pH 3-4), exhibiting the adaptability of the species towards acidic stress. The amelioration of the soil fertility (increased of pH and EC) resulted in the significantly highest concentration of Fe in *M. malabathricum* in T4, suggesting that this species could be a suitable candidate for the EK-assisted phytoremediation in a real acidic slope condition with a careful operating condition should be selected in order to optimize the phytoextraction and minimize the inhibition of the plant growth (Cameselle et al., 2013). Whilst for the Al, we discovered that the *M. malabathricum* and *S. campanulatum* did not respond to the EK-assistance by having similar Al contents in their plant parts, perhaps due to their characteristic which proven as Al-accumulator plants.

Apart from that, the low BCF and TF values (less than 1) calculated for both species indicates that *M. malabathricum* and *S. campanulatum* are non-hyper-accumulator species encountering the excess amounts of Fe and Al. However, it is evident that the total accumulation of more than 1000 mg/kg of Fe and Al recorded in their plant parts show the effective contribution of EK in metals bioavailability in soils as well as their suitability as phytoremediation candidates.

In regards to the above findings, we discover that the EK-assistance has provide better outcome in improving soil acidic conditions through various mechanism adopted by the selected species to accumulate, translocate, and detoxify metals. The soil pH becomes less acidic by 2.7-4.6%, verifying the importance of electric current to accelerate the metals mobility and enhance the bioavailability in soil, subsequently contributes to the soil fertility improvement. Moreover, the large amounts of metals in bioavailable forms lead to a notable concentration of targeted metals, Fe and Al in the plant parts of *M. malabathricum* and *S. campanulatum*. Hence, the combination of phytoremediation with electro-kinetic remediation is beneficial not only for remediating the acidity problem of the areas with similar condition but also could help to protect the slope soils against the erosion.

Conclusion

This study exhibits an assessment of tolerance and suitability of the species studied for phytoremediation potential. It is evident that electro-kinetic application is a promising technique for the enhancement of bioavailability of the metals in soil for the plants uptake, especially in acidic condition. A combination of EK with phytoremediation has positively improved the soil pH and conductivity level in soil, thus, alleviating the soil acidity. Although the plant growth performance has shown adverse effect of EK-assisted treatment, the selected acidic-tolerant slope plants able to accumulate excessive amount of iron (Fe) and aluminium (Al) in their plant parts. However, the results warrant further trials on the field to truly establish the practicality of utilizing both species as phyto-remediator with the assistance of electro-kinetic.

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