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# Electrokinetic Enhancement on Phytoremediation in Zinc Contaminated Soil by Ruzi Grass

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**Abstract**

The use of Ruzi grass (*Brachiaria ruziziensis*) for electrokinetic (EK)-phytoremedial and phytoremedial removal of zinc ions ($Zn^{2+}$) from contaminated soil was demonstrated in a laboratory-scale experiment. After 15 d of germination, Ruzi grass seedlings were transferred to experimental soil pots that were supplemented to 0, 300, 400 and 500 mg $Zn^{2+}$/kg soil. After 15 d growth, the $Zn^{2+}$ concentration that allowed the highest survival rate and biomass of Ruzi grass was selected to sequentially determine the optimum applied voltage (from 0, 1, 2 and 4 V/cm) and then the duration of the selected applied voltage (0, 2, 4 and 6 h/d). An applied voltage of 2 V/cm for 2 h/d was found to be the most optimal for Zn accumulation in the Ruzi grass, and this was then used to treat soil contaminated with a high concentration of $Zn^{2+}$ (1000 mg $Zn^{2+}$/kg soil) in comparison with and without the applied electric field (*phytoremediation*) over a 15 d treatment. The EK-phytoremediation significantly increased the accumulation of zinc (Zn) in Ruzi grass roots (but not shoots) and decreased the residual Zn levels in the soil compared to that with phytoremediation only. The plant Zn concentration following EK-phytoremediation (393.8 ± 19.7 mg/kg) was almost 4.4-fold higher than that in the phytoremediation system (89.9 ± 4.5 mg/kg).

***Keywords:*** electrokinetic; phytoremediation; soil; zinc; Ruzi grass

## 1. Introduction

Phytoremediation is a process in which green plants are used to reduce the toxicity of contaminants in soil, water and air. Phytoextraction is the uptake of heavy metals (Cd, Cu, Cr, Ni, Zn, Pb, Co, Mn and Hg) in plant tissue (Vamerali *et al*., 2010). The advantages of phytoremediation are the low cost of operation, convenience and its environmentally friendly nature (Putra *et al*., 2013), while its limitations are the penetration depth of the roots into the soil, a low biomass production and a slow growth rate (Ali *et al*., 2013). The bioavailability of the contaminant(s) can be increased by the application of chelating agents (Wu *et al*., 2007) and surfactants (Gao *et al*., 2008). Furthermore, the application of an electric field was found to help increase the absorption of metals by plants (Cameselle *et al*., 2013). Aboughalma *et al*. (2008) reported that the ability of potato plants to reduce heavy metal ion levels in contaminated soils was enhanced with an applied electric field. They found an applied alternating current (AC) promoted the growth of plants and increased the heavy metal accumulation better than an applied direct current (DC). For tobacco and rapeseed growing in contaminated soil, an applied electric field enhanced both their biomass production and heavy metal uptake, but an applied DC field tended to induce a pH change in the soil and provoke metal redistribution, a negative influence, more than an applied AC field (Bi *et al*., 2011).

Zinc (Zn) deficiency in human diets has recently received increasing attention (Cakmak, 2009). The synergism between phytoremediation of contaminated soil and enhancing the concentration of micronutrients in food crops has been studied (*Zhao and McGrath*, 2009). Phytoextraction may provide an effective tool for biofortification of food crops via increasing the concentration of zinc in food plants and the food chain thereafter (Cakmak, 2008). Ruzi grass (*Brachiaria ruziziensis*) is a forage crop that is a hyperaccumulator of heavy metal ions (*Sripraisont*, 2005), and so could be appropriate to use in the biotreatment of heavy metal ion contaminated soil. Ruzi grass grows well in tropical climates with annual rainfall of over 1000 mm/y, such as in Thailand, and also it is tolerant to environmental changes and is easy to handle.

Thus, the objectives of this study were to investigate the concentration of Zn ions ($Zn^{2+}$) that Ruzi grass can tolerate, and the optimum applied AC voltage and duration of application for its growth and $Zn^{2+}$ uptake (accumulation) from the soil.

## 2. Materials and Methods

### *2.1. Soil sampling and preparation*

The soil sample was collected from an orchard area in Chainat province, Thailand from a depth of 0-20 cm from the surface. The soil was put into a polyethylene bag and transported to the laboratory, where it was divided into two parts. The first part was air-dried, crushed and sieved through 2.0 and 0.5 mm and then analyzed for its physical and chemical properties by the methods listed in Table 1, with the results also summarized in Table 1.

The physical and chemical properties of the soil sample were appropriate for electrokinetic studies. The soil samples were clay in texture with a high Cation Exchange Capacity, and so $Zn^{2+}$ will be able to bind to the clay particles in neutral soil. However, the pH of the soil used in this study was low (5.9), and so heavy metal ions like $Zn^{2+}$ would be easy to dissolve and so result in an enhanced phytoremediation level.

The other soil part was supplemented with $Zn^{2+}$ (as $ZnSO_4.7H_2O$) at 0, 300, 400 and 500 mg $Zn^{2+}$/kg soil. These values were selected from the results of a preliminary study on the effect of $Zn^{2+}$ (100-800 mg $Zn^{2+}$/kg soil), where at 100-300 mg $Zn^{2+}$/kg soil, the survival and appearance of Ruzi grass was not affected, at 400-500 mg $Zn^{2+}$/kg soil the survival was good but the plants appearance was altered and at 600-800 mg $Zn^{2+}$/kg soil the plant survival was markedly reduced. The soil (1 kg/pot) was packed into rectangular glass pots of 14 (L) x 12 (W) x 10 (H) cm, giving a thickness of the soil layer in the pots of ~ 6 cm.

### *2.2. Ruzi grass preparation*

Seeds of Ruzi grass were purchased from Praweenporn farm in Tak province, Thailand. Some of the seeds were sent to the seed association of Thailand to confirm the species identification as Ruzi grass, whilst the rest were pregerminated for 15 d in a growth chamber of 40 (L) x 40 (W) x 10 (H) cm. After 15 d the seedlings were carefully selected to provide a more uniform distribution of shoot and root biomass (estimated by size) and then washed to remove the soil before being placed in the experimental pots.

### *2.3. Experimental details*

#### *2.3.1. Determination of the highest survival rates and biomass*

The 15-d-old Ruzi grass seedlings (section 2.2) were transferred to the experimental soil pots (10 seedling/pot) supplemented with $Zn^{2+}$ at 0, 300, 400 and 500 mg $Zn^{2+}$/kg soil and allowed to grow for 15 d. The plants were then harvested, washed by tap water followed by deionized water and the shoots and the roots were separated, weighed (wet weight) and then dried in an oven for 2 d at 65°C and reweighed (dry weight). From this the concentration of added $Zn^{2+}$ to the soil that resulted in the greatest biomass of Ruzi grass was derived.

#### *2.3.2. Determination of the optimum applied voltage level*

The $Zn^{2+}$ contaminated soil that gave the highest survival rate and biomass of Ruzi grass (section 2.3.1) was then used to determine the optimum applied voltage in the EK-phytoremediation. The Ruzi grass seedlings were planted in this soil and grown for 15 d as otherwise described in section 2.3.1 except that an AC electric field was applied from two pairs of electrodes (Fig. 1(A)) positioned so as to divide the pot into an equal sized upper anode and lower cathode region (Fig. 1(B)). The electrodes were graphite rods (6 cm in length and 2 mm in diameter) vertically inserted in the experimental pot

Table 1. The physical and chemical properties of the soil used in this study, and the analysis method used

| Parameter | Analytical method | Value | Unit |
|---|---|---|---|
| Soil texture | Hydrometer method | Clay | - |
| Particle size distribution | Hydrometer method | Sand: Silt: Clay = 39:19:42 | % |
| Water holding capacity | Gravimetric method | 44.5 ± 2.30 | % |
| pH | pH meter (soil: water = 1:2.5 (v/v)) | 5.9 ± 0.09 | |
| Organic matter content | Walkley & Black method | 2.20 ± 0.07 | % |
| Cation Exchange Capacity | $NH_4OAc$ method | 36.4 ± 1.90 | $cmol_c$/kg |
| Total Nitrogen | Kjeldahl method | 0.8 ± 0.16 | % |
| Available Phosphorus | Mehlich 1 method | 0.3 ± 0.01 | mg/kg |
| Available Potassium | $NH_4OAc$ method | 2.4 ± 0.03 | mg/kg |
| Total $Zn^{2+}$ | EPA method 3052 | 71.3 ± 10.8 | mg/kg |

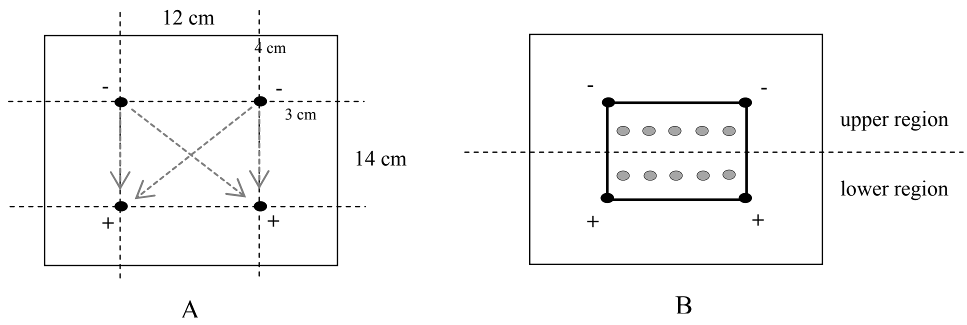


Figure 1. Schematic diagram of the electrokinetic (EK)-phytoremediation system

to a depth of 4 cm below the surface. Each treatment was performed in triplicate with random allocation of the pots to each treatment. The temperature was maintained by an air conditioner at 27°C. The soil moisture content in all pots was controlled at 70 % water holding capacity by weight. An applied voltage (0, 1, 2 and 4 V/cm) was applied for 15 d at 2 h/d and then the plants were harvested, washed and the shoot and root wet and dry weights were determined as in section 2.3.1. In addition, the dried root and shoot powders were screened for their total Zn content by digesting with concentrated $HNO_3$ in a microwave digester (Ethos one, ACT36–Rev01–03/06 Model, Italy) following EPA method 3052 (US EPA, 1996) followed by atomic absorption spectrophotometry with a SpectrAA 200 (Agilent, USA) instrument.

*2.3.3. Determination of the optimum duration of the applied voltage.*

The optimal applied $Zn^{2+}$ concentration in the soil (section 2.3.1) and applied voltage (section 2.3.2) were then used to determine the optimum duration of the applied voltage. Ruzi seedlings (15 d old) were transplanted to the experimental soil and grown for 15 d as detailed (sections 2.3.1 to 2.3.2) with the duration of the applied AC voltage (2 V/cm) being varied at 0, 2, 4 and 6 h/d for the 15-d period. They were then harvested, washed and determined for their shoot and root wet and dry weights and Zn content as described in section 2.3.2.

*2.3.4. Comparison between phytoremediation and EK-phytoremediation using soil contaminated with a high concentration of zinc (1000 mg $Zn^{2+}$/kg soil)*

The soils were artificially amended with $Zn^{2+}$ at 1000 mg $Zn^{2+}$/kg soil (the level often found in soil near zinc mining areas) and the 15-d old Ruzi seedlings (section 2.3.1) were planted in the soil and grown for 15 d without an applied electric field (phytoremediation) or with an applied AC field of 2 V/cm at 2 h/d (EK-phytoremediation). Each treatment was performed in triplicate. After 15 d the Ruzi grass was harvested, washed and determined for the shoot and root wet and dry weights and the Zn content as described in section 2.3.2.

*2.4. Statistical analysis*

All the data were analyzed using the SPSS version 22 (Statistical package for the social science for windows) software using a One-way ANOVA followed by Turkey's HSD (Honestly Significant Difference) test, with significance being accepted at the $p < 0.05$ level.

## 3. Results and Discussion

*3.1. Survival rate and biomass production*

The survival rate and biomass of Ruzi grass were numerically decreased with increasing applied concentrations of $Zn^{2+}$ in the soil, but the survival rate and biomass of Ruzi grass grown in 300 mg $Zn^{2+}$/kg soil was not significantly different to that in the uncontaminated soil (Fig. 2). However, the survival rate and biomass of plants grown in soil supplemented with 400 and 500 mg $Zn^{2+}$/kg soil were significantly lower than those grown with 0 and 300 mg $Zn^{2+}$/kg soil. Zinc toxicity can occur in crops growing on an acidic soil enriched by anthropogenic Zn inputs (Broadley *et al*., 2007), where an excess supply of Zn affects both the shoot and root growth, with the shoots becoming stunted and chlorotic (Pahlsson, 1989). Therefore, a $Zn^{2+}$ concentration of 300 mg $Zn^{2+}$/kg soil was selected.

*3.2. Effect of the voltage level on zinc accumulation in Ruzi grass*

The level of Zn accumulation in the shoots and roots of Ruzi grass increased slightly with increasing applied voltage up to 2 V/cm and then decreased slightly at 4 V/cm, but none of these numerical differences

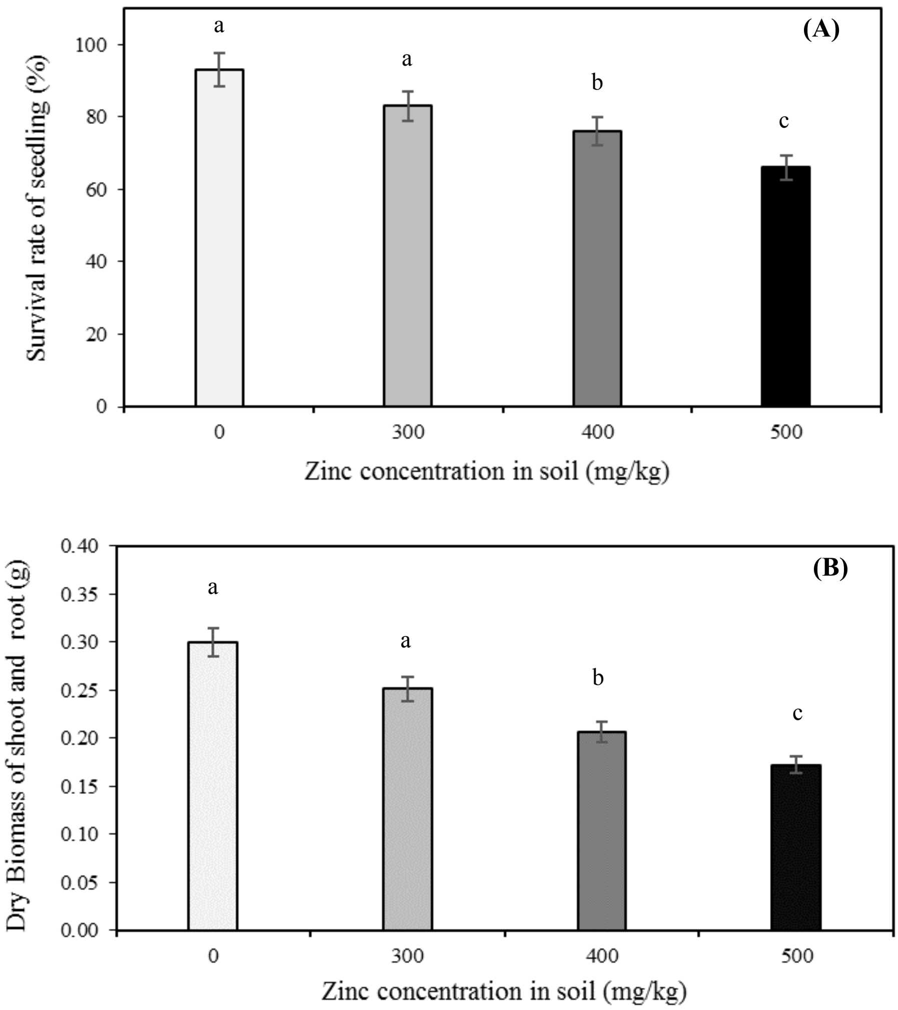


Figure 2. The (A) survival rate and (B) biomass of Ruzi grass grown in soil supplemented with 0, 300, 400 and 500 mg $Zn^{2+}$/kg soil for 15 d. Data are shown as the mean ± 1 S.D., derived from three independent repeats. Means with a different letter are significantly different ($p < 0.05$)

were significant (Fig. 3(A)). However, the level of Zn accumulation was significantly higher in the roots than in the shoots in all cases (Fig. 3(A)). Re-evaluation of the data in terms of the zinc uptake per pot (mg Zn/pot) did not reveal any significant differences (not shown). Since an applied voltage of 2 V/cm caused the highest accumulation of Zn in the shoots and roots, it was selected as the optimal applied voltage. In accord with these results, it was previously reported that a low voltage enhanced the growth and development of Indian mustard but a high voltage (4 V/cm) had a slightly adverse effect on the Cd, Cu and Zn accumulation in roots (Cang *et al*., 2011). The application of an AC field led to the removal of heavy metal ions from the soil and their accumulation in potato roots and shoots, but mainly in the roots, at a higher level than the control without an applied electric field (Aboughalma *et al*., 2008).

### *3.3. Effect of the duration of the applied voltage*

Zinc accumulation in the shoots and roots of Ruzi grass increased markedly with a 2 h/d applied electric field but then decreased with longer daily durations such that a 6 h/d EK-phytoremediation was no better than that with phytoremediation only (Fig. 3(B)). Re-evaluation of the data in terms of the zinc uptake per pot (mg Zn/pot) did not reveal any significant differences (not shown). However, none of these were significantly different except for the Zn accumulation in the roots with a 2-h/d duration of the applied electric field that was significantly higher. The Zn accumulation level in shoots and roots at an applied voltage of 2 V/cm for 2 h/d were 929 ± 37 and 1,551 ± 81 mg/kg, respectively, after 15 d. In this study, since the soil water contents were kept constant in each treatment by the gravimetric method, then the applied voltage mainly controlled the

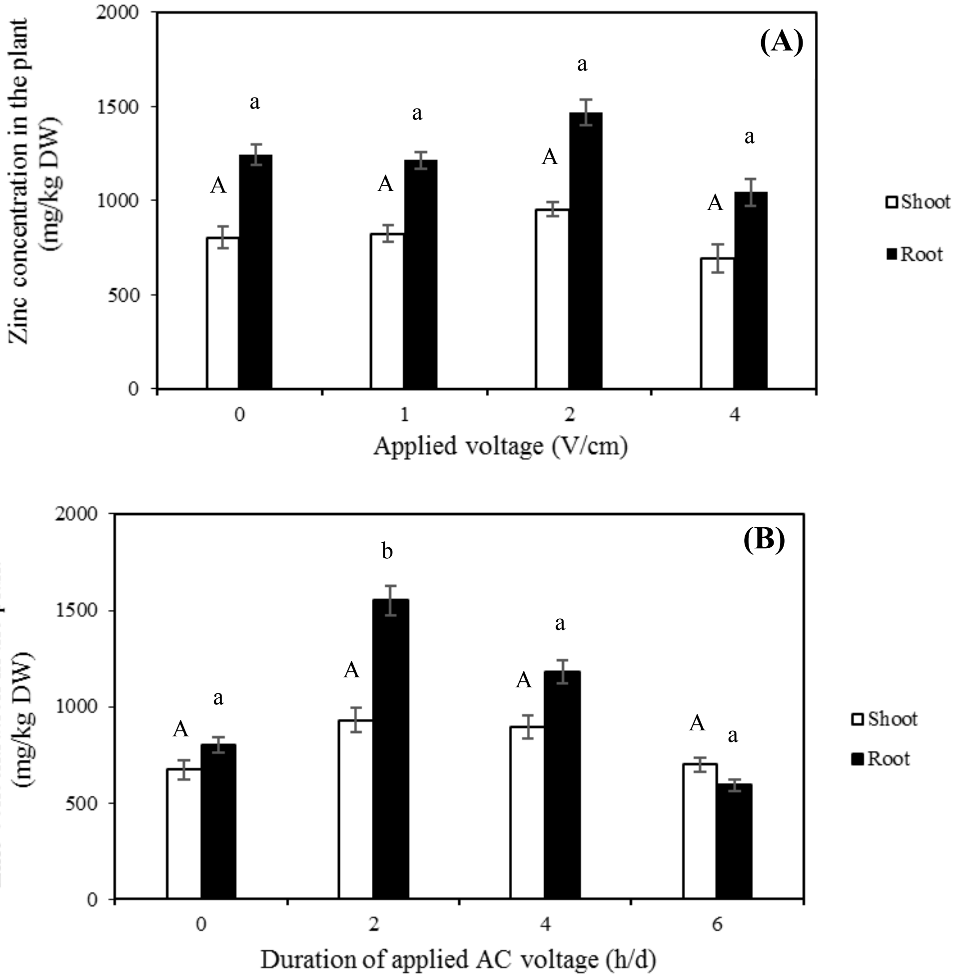


Figure 3. Zinc accumulation in the shoots and roots (as dry weight; DW) of Ruzi grass at (A) varying applied AC voltages for 2 h/d for 15 d and (B) with different periodic (h/d) applied AC voltages of 2 V/cm. Data are shown as the mean ± 1 S.D., derived from three independent repeats. Means with a different letter (capital for within shoots and lowercase for within roots) are significantly different ($p < 0.05$)

electric current. Previously, a vertical DC electrical field of 1 V/cm applied at 6 h/d for 7 d was found to significantly increase the ryegrass uptake of Cu/Zn compared with the control when applied with the chelating agents ethylenediaminetetraacetic acid and ethylenediamine-N, N'-disuccinic acid (Zhao *et al*., 2007). In addition, Lim *et al*. (2004) found that the periodic application of an electric potential for 9 d at 1 h/d resulted in better metal uptake than at 0.5 h/d in Indian mustard. In this study, an applied AC voltage level of 2 V/cm for 2 h/d was selected as the more suitable condition.

### *3.4. Comparison between phytoremediation and EK-phytoremediation for the uptake of $Zn^{2+}$ from a high $Zn^{2+}$ soil concentration (1000 mg $Zn^{2+}$/kg soil)*

Ruzi grass seedlings were grown for 15 d in soil supplemented with a high concentration of $Zn^{2+}$ (1000 mg $Zn^{2+}$/kg soil) without (phytoremediation) or with an applied AC voltage of 2 V/cm at 2 h/d (EK-phytoremediation) and then the Zn levels were ascertained and compared (Fig. 4). Re-evaluation of the data in terms of the zinc uptake per pot (mg Zn/pot) did not reveal any significant differences (not shown). The results showed that the zinc accumulation in the shoots showed no significant difference between phytoremediation and EK-phytoremediation, whereas the Zn accumulation in the roots was significantly (4.38-fold) higher with EK-phytoremediation (393.8 ± 19.7 mg/kg) than with phytoremediation (89.9 ± 4.5 mg/kg). Thus, the electric field enhanced the ability of plant roots to absorb $Zn^{2+}$ from the contaminated soil. This result concurs with the study of Aboughalma *et al*. (2008), where metal accumulation in the plant roots was enhanced by the application of an electric field. Moreover, Cameselle *et al*. (2013) reported that an applied electric field may enhance metal ion extraction by increasing their bioavailability via promoting their desorption and transport, even over short distances.

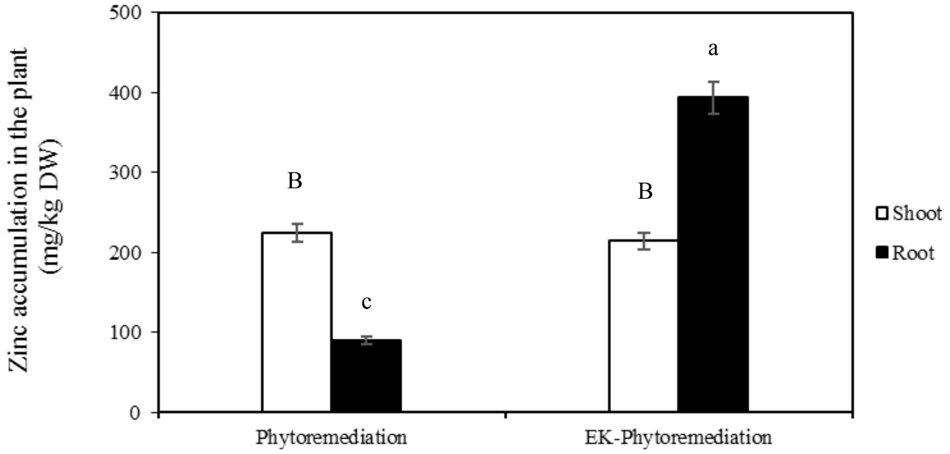


Figure 4. Zinc accumulation in the shoots and roots of Ruzi grass grown for 15 d in soil with 1000 mg $Zn^{2+}$/kg soil without (phytoremediation) or with (EK-phytoremediation) an applied AC electric field of 2 V/cm at 2 h/d. Data are shown as the mean ± 1 S.D., derived from three independent repeats. Means with a different letter (capital for within shoots and lowercase for within roots) are significantly different (*p < 0.05*).

The residual $Zn^{2+}$ levels in soil after the 15 d phytoremediation and EK-phytoremediation were slightly higher in the cathode (lower) region than in the anode (upper) region (Fig. 1(B)) of the soil surface (Fig. 5), but these differences were only significant in the case of the EK-phytoremediation. The residual $Zn^{2+}$ levels in cathode region of the soil following phytoremediation and EK-phytoremediation were 278 ± 10 and 215 ± 12 mg/kg, respectively. In both regions, the EK-phytoremediation decreased the $Zn^{2+}$ level in the soil more than phytoremediation, which is presumably due to the electric field mobilizing $Zn^{2+}$ and accumulating them in the cathode area as the main factor affecting the distribution of $Zn^{2+}$ in the soil. In agreement, Aboughalma *et al.* (2008) found that under EK-phytoremediation of heavy metal contaminated soil, the heavy metal migration was from the anode site to the cathode site.

### *3.5. Short- and long-term studies*

It is important to note that in this study the EK-phytoremediation of Zn from soil by Ruzi grass involved soil that had only recently been contaminated with $Zn^{2+}$. As such this at best represents an experimental model of recently contaminated soil, which may well differ from longer term contaminated soil where the complex interactions leading to more complete absorption or chelating of $Zn^{2+}$ will have had time to be achieved. Thus, long-term experimental systems, such as preseeding the soil with $Zn^{2+}$ for 3-6 months before planting the Ruzi grass, are required.

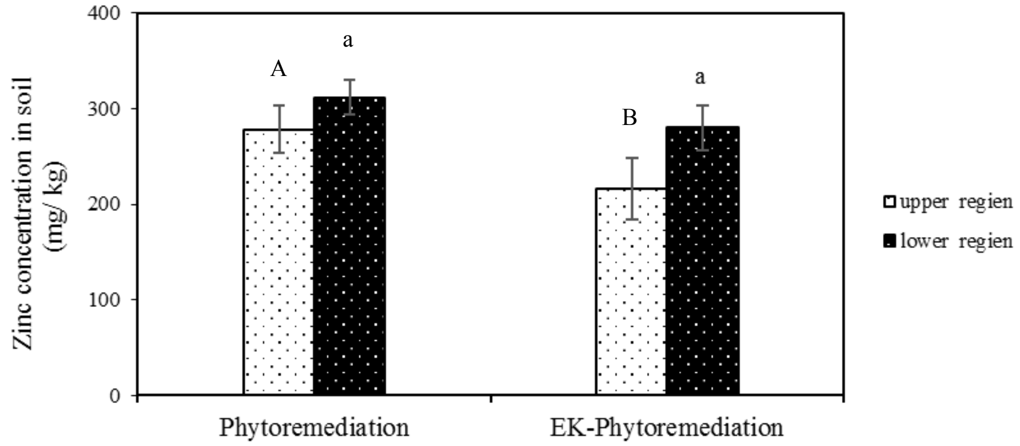


Figure 5. Comparicson of zinc levels in the soil after phytoremediation and EK-phytoremediation (2 V/cm AC current at 2 h/d) by Ruzi grass for 15 d. Data are shown as the mean 1 S.D., derived from three independent repeats. Means with a different letter (capital for within upper and lowercase for within lower region.) are significantly different *(p<0.05)*

## 4. Conclusions

The significant variables that affect EK-phytoremediation are the use of an AC or DC current, the applied voltage level and the mode of voltage application in terms of continuous or periodic and, for the latter, its duration (Cameselle *et al*., 2013) In this study, the optimum condition for Zn accumulation in Ruzi grass was an applied AC voltage of 2 V/cm for 2 h/d, where the $Zn^{2+}$ could be mobilized with only a minor effect on the plant growth. Zinc accumulation in the roots of Ruzi grass was greater than in the shoots under this applied periodic electric field. Ruzi grass is a suitable plant for treatment of soil contaminated with $Zn^{2+}$ because of its tolerance to high $Zn^{2+}$ concentrations and ability to accumulate Zn in its tissues. However, the optimum condition for each heavy metal and plant cultivar/species should be ascertained for increasing the efficiency of EK-phytoremediation, as well as in long-term contaminated soils.


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