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ORIGINAL PAPER

Alternating current electrical field effects on lettuce *(Lactuca sativa)* growing in hydroponic culture with and without cadmium contamination

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Abstract The aim of the present study was to investigate the effect of a 10 Hz alternating current (10 Hz 1 V cm^{-1}) and a 50 Hz alternating current (50 Hz 1 V cm^{-1}) on the lettuce plant (*Lactuca sativa*) growing in a hydroponic (soil-free) culture. Thirty lettuce plants were pre-germinated, and then 15 of them were treated with cadmium solution ($CdCl_2$) of 5 mg/L in concentration. Ten plants (five plants with Cd and five plants without Cd) were subjected to a 10 Hz alternating current (AC) electrical field; 10 plants were subjected to a 50 Hz AC field. The rest of the plants were used as a control. The lettuce plants were harvested after a growth of 60 days. The chlorophyll content, biomass and metal content of the lettuce plants were determined. The biomass of the plants growing in non-contaminated medium was 28 and 106% higher under the 10 and the 50 Hz AC fields respectively compared to the control. Although the plant biomass was reduced by the presence of Cd in the growth medium, the biomass of the plants growing in Cd contaminated medium was 40 and 63% higher respectively for 10 and 50 Hz AC field compared to the plant growing in Cd contaminated medium without electrical treatment. Increased uptake of Cd in the plant shoot was found with the 50 Hz AC field. Significant accumulation and uptake of Cu in plant roots and shoots was found under both electrical treatments.

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1 Introduction

There has been increasing interest in combining electrokinetic techniques together with phytoremediation in contaminated soil treatment. However, this was mostly the application of a low voltage direct current (DC) field used in electro-bioremediation and electro-phytoremediation [1–4]. Several studies have demonstrated improved biodegradation of organic pollutants under the influence of a low voltage DC field, due to the induced movement of water, nutrients, contaminants and microorganisms, which led to an overall homogenization of the contaminated soil [2, 5]. It was noted by O'Conner et al that the application of a DC field stimulated the growth and metal uptake of ryegrass growing near the cathode region, and the plant growth was slightly lower near the anode [3]. Moreover, a reduced growth of potato plants in a multi metal contaminated soil was found by Aboughalma et al. [4], due to the acidic soil conditions created by the DC field. Conversely, a low voltage 50 Hz AC field significantly enhanced biomass production for potato plants growing in the multi metal contaminated soil was found, and no other unfavorable soil conditions were found under the 30 days AC treatment. However, the origin of the interaction of a low voltage AC electrical field on the plant itself was unknown.

Plants consist of living cells, which functioned by their electrophysiology. The plasma membrane of the plant root is normally negatively charged. There are self-generated electric fields and currents in the energetic metabolism of plants [6, 7]. It can be assumed that an external electric field might also have an effect on the plant. There are two major transport processes in the plants: the active and the passive transport processes. Active transport is the process by which organisms take up minerals against a concentration gradient, and this process requires energy. Passive transport is the transport process caused by the concentration gradient across the plant root membrane, and this process requires no energy [8]. The cation channels in the plant are macromolecular protein pores in bio-membranes that catalyze passive cation influx and efflux, which they do not use ATP energy to transport cations [9]. Cation channels play multiple physiological roles in plants. They catalyze nutritional uptake of N (taken up as NH_4^+), macronutrient and micronutrient cations, such as K^+, Ca^{2+}, Na^+, Fe^{2+}, Cu^{2+}, Ni^{2+}, Co^{2+}, Zn^{2+} and Mn^{2+}. Cation channels are responsible for the generation of negative action membrane potentials [10].

The first large scale experiment of the electric field influences on the plant was carried out by Selim Lemström in the early twentieth century. He imitated the weak electric currents carried through the atmosphere by air ions with an electrostatic generator. The voltage gradient was approximately 10 kVm^{-1}. It was found that the treated plants were greener, sturdier and often showed a dramatic increase in yield [11]. The electric field could enhance the germination rate of the plant [12, 13], as well as stimulate the plant growth [14, 15]. The influence of the electric field could last for some time, even after the current was switched off, which could also be an explanation why the plants in nature look unusually green after thunderstorms [16]. Murr also found out that the plants seem to be greener under the influence of a higher voltage electrical field, which was similar to the thunderstorms in the nature [17].

The aim of the present experiment was to investigate the effect of a low voltage AC field at 10 and 50 Hz on the lettuce plants growing in a soilless hydroponic culture, and the response of plant chlorophyll content, metal content and biomass production.

2 Materials and methods

2.1 Germination and pre-growth of the plants

Lettuce (*Lactuca sativa*) was used in this experiment, due to its ability to grow in the liquid system, and relative tolerance to heavy metals. The plant seeds were first exposed to the sun in dry environments to improve the germination rate. The germination process was carried out in an incubator (Snijders Scientific B.V., NL). The temperature was set to 24 °C in the day time and 18 °C in the night. The day and night cycle was 16 and 8 h, respectively. The plant seeds were soaked in distilled water for 24 h before being placed in the petri dish. Two layers of filter paper were used in the petri dish with 3 mL of distilled water to maintain moist condition during germination. The germination on the filter paper took 4 days. When the plants developed 2 cotyledons, they were transplanted into a pre-growth vessel (15 × 45 × 5 cm) containing 800 mL of 1:10 diluted micro-and macro-elements nutrient solution (Table 1). The baby plants were fixed within a piece of 8 mm thick Styropor (15 × 45 cm) with equally distributed holes on it (diameter 3 mm), one plant placed in each hole. The Styropor was floated on the diluted nutrient solution, with the plants roots suspended in the liquid nutrient, and the plant shoots above the Styropor (Fig. 1).

Table 1 The concentration of the micro- and macro-elements in the growth medium (Murashige & Skoog Medium M0254, Duchefa Biochemie B.V., NL)

Micro-elements	[mg/L]	Macro-elements	[mg/L]
$CoCl_2 \cdot 6H_2O$	0.025	$CaCl_2$	332.02
$CuSO_4 \cdot 5H_2O$	0.025	KH_2PO_4	170.00
FeNaEDTA	36.70	KNO_3	1900.00
H_3BO_3	6.20	$MgSO_4$	180.54
KI	0.83	NH_4NO_3	1650.00
$MnSO_4 \cdot H_2O$	16.90		
$Na_2MoO_4 \cdot 2H_2O$	0.25	Buffer	[mg/L]
$ZnSO_4 \cdot 7H_2O$	8.6	MES	4905.19

2.2 Plant growing in the liquid nutrient medium

The lettuce plants with similar size and development were selected and transplanted to individual growing pots (10 × 11 × 8 cm) when they had 4 or 5 leaves. The transparent plastic growing pots were made by Duchefa Biochemie B.V. NL, specially designed for plant growth. A thick black plastic film was used to cover the pots, which prevented the plant roots being exposed to the sun light during growth. Each plant was fixed with a piece of 8 mm thick Styropor on top of the pots. Small holes in the Styropor allowed for ventilation. Oxygen supply for the plant roots was achieved by a commercial fish tank pump. A volume of 750 mL of 100% nutrient solution (Table 1) was used in each pot. After transplanting, cadmium ($CdCl_2$ 99.99%) was added to 15 of the pots, giving a Cd concentration of 5 mg/L. The other 15 pots were not treated with Cd. An AC electrical field (1 V cm^{-1}) was applied with different frequencies, in 10 pots (five pots with Cd and five pots without Cd) a frequency of 10 Hz and in 10 pots a frequency of 50 Hz were applied. Ten pots were without electrical treatment, which remained as control for both Cd contaminated and non-contaminated nutrients. All the plants used in this step were with a similar size. The temperature of the nutrient solution in all the pots was kept constant. Distilled water was used every 2 or 3 days for

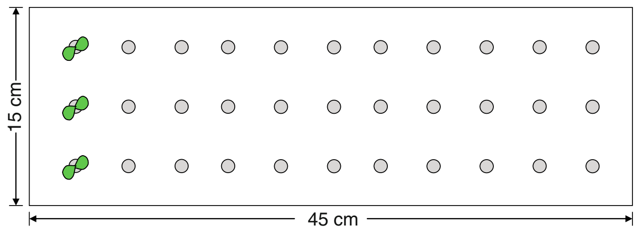

Fig. 1 Pre-germination set up of baby plants on a piece of Styropor

filling up the pot to the original volume. Nutrient concentration, such as nitrate and phosphate were randomly determined during the experiment, and a concentrated nutrient solution was added during the experiment to maintain a constant nutrient supply for the plant.

2.3 Chlorophyll analysis

Before harvesting the plants, the intermediate aged plant leaves were chosen for chlorophyll determination. Leaf discs were punched out from the plant leaves using a cork borer (diameter 8 mm), and then immediately frozen at −24 °C. The chlorophyll extraction was performed by taking 1 piece of leaf disc dissolved in 4 mL of 100% acetone. The extract was made up to a volume of 4 mL and then centrifuged for 8 min at 1,500 rpm to get a clear solution. The absorbance of the extract was determined by a spectrophotometer (Lambda 2 UV/VIS, Perkin Elmer) at a wavelength of 470, 644.8, 661.6 and 750 nm, and the chlorophyll content was calculated using the method of Lichtenthaler [18].

2.4 Biomass and metal content in plants

After a total of 60 days growth, the lettuce plants were harvested and the fresh mass of the plants was measured with an analytical balance. Then, the plants were dried in the oven at 75 °C for 48 h, and the dry mass of the plants was recorded after drying. The shoot and root of each plant were ground separately. About 0.5 g of each ground plant sample was digested with 10 mL of HNO_3 and 2 mL of H_2O_2 in a closed digestion vessel by a microwave digestion unit (MARSXpress, CEM Corporation, USA). After cooling, the digested solution was filtered and made up to a volume of 50 mL. The metal content in the plant sample was determined by using a flame atomic absorption spectrometer (SpectrAA 220 FS, Varian, USA).

3 Results and discussions

3.1 Chlorophyll content

Photosynthesis is the basis of the plant metabolism, and chlorophyll is the primary substance of light energy absorption and transformation. Therefore, the factors that affect the chlorophyll content would have a direct effect on the plant growth [19]. By applying the low voltage alternating current fields, both of the chlorophyll *a* and chlorophyll *b* content in the plant were increased compared to the plants without electrical treatment. The chlorophyll *a* content under AC 10 Hz and 50 Hz was 19 and 36% higher respectively compared to control (Fig. 2). The chlorophyll *b* content under AC 10 Hz and 50 Hz was 40 and 88% higher comparing to the control (Fig. 3). The carotenoids content was also enhanced under the influence of the AC field: the carotenoids content was 11% higher and 23.3% higher for AC 10 and 50 Hz compared to the control (Fig. 4).

Chlorophyll is the most important type of plant photosynthetic pigment and its content in the plant can decrease when the plant is under a stress [20]. The toxicity of the induced cadmium introduced to the growth medium led to leaf chlorosis of the lettuce plant [21], which was observed during the experiment. It can be seen in Figs. 2, 3 and 4 that the presence of Cd in the growth medium diminished the chlorophyll generation in the plants (chlorophyll *a*, chlorophyll *b*, carotenoids). However, higher chlorophyll content was still found in the plant treated with the

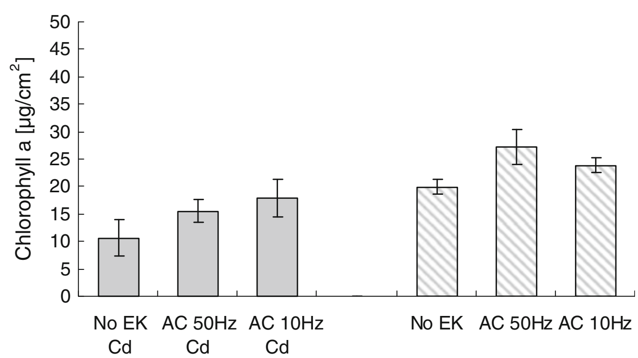

Fig. 2 Chlorophyll *a* content of plants growing in non-contaminated and Cd contaminated solutions under three treatment conditions

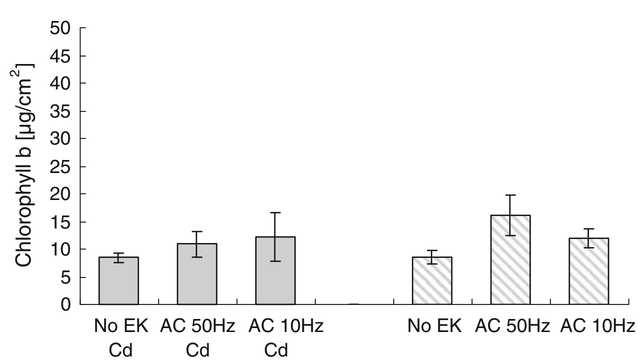

Fig. 3 Chlorophyll *b* content of plants growing in non-contaminated and Cd contaminated solutions under three treatment conditions

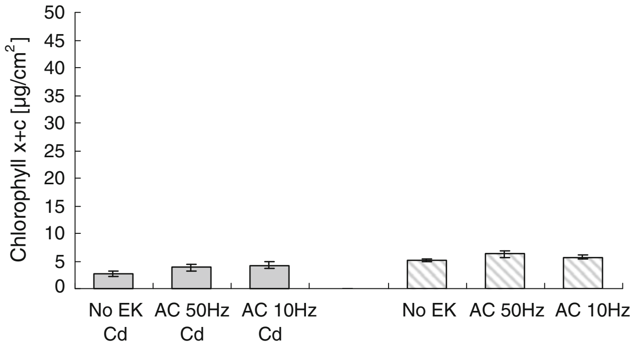

Fig. 4 Carotenoids content of plants growing in non-contaminated and Cd contaminated solutions under three treatment conditions

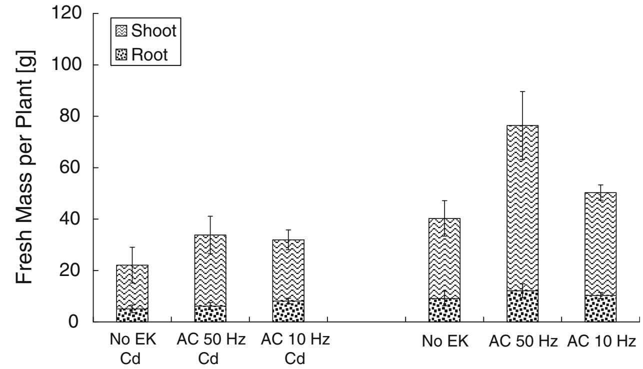

Fig. 5 Fresh shoot and root mass of lettuce plants growing under three treatment conditions

electrical field. The chlorophyll *a* was 67% higher in the plant treated with AC 10 Hz and 31% higher in the plants treated with AC 50 Hz in the Cd contaminated growth medium in contrast with the control plant (Fig. 2). The chlorophyll *b* in the plant was 45% higher under AC 10 Hz and 29% higher under AC 50 Hz comparing to the control (Fig. 3). The carotenoids were 56 and 40% higher for AC 10 Hz and 50 Hz than control in the Cd contaminated solution (Fig. 4).

3.2 Biomass

The plant contains an internal electrical field [7], so an external electrical field may alter the pattern of metabolism in the plant, as well as altering the passive transport process of micro- and macro-elements via the cation channels. Cation channels play multiple physiological roles in plants. They catalyze nutritional uptake of N (taken up as NH_4^+), macronutrient and micronutrient cations, such as K^+, Ca^{2+}, Na^+, Fe^{2+}, Cu^{2+}, Ni^{2+}, Co^{2+}, Zn^{2+} and Mn^{2+}. Cation channels are responsible for the generation of a negative membrane voltage and action potentials [10]. The current experiment with the AC electrical field of 10 and 50 Hz showed an enhancement of the biomass, especially under the 50 Hz AC field in the non-contaminated nutrient solution (Fig. 5). By the application of the electrical field to the growth medium, the root growth of the plants was stimulated (Fig. 5), which given a root mass of 10.3 ± 1.2 g, 12.1 ± 2.7 g and 9.1 ± 2.8 g for AC 10 Hz, AC 50 Hz and control, respectively. A diminished root growth was found in the presence of Cd in the growth medium, with a root mass of 8.1 ± 1.0 g, 6.2 ± 1.3 g, 5.1 ± 1.4 g for AC 10 Hz, AC 50 Hz and control, respectively. The plasma membrane of the plant root cell is often negatively charged [7], so the external electrical field might increase the affinity of the charged ions to the membrane including minerals and nutrients. The increased root growth under the electrical field could enable a higher transport of nutrient from the root to the shoot, which further enhances the shoot mass for a higher trans-evaporation rate of the plant [3]. The shoot mass of the plant growing in non-contaminated nutrient under AC 10 Hz was 40 ± 3.0 g, under AC 50 Hz was 64.3 ± 13.2 g and for the control was 31.2 ± 6.9 g (Fig. 5). The shoot mass of the plant growing in the Cd contaminated nutrient under AC 10 Hz was 23.8 ± 3.9 g, under AC 50 Hz was 27.7 ± 7.3, and under control was 17 ± 7.0 g (Fig. 5). Thus the introduction of Cd to the growth medium could prove toxic to the plant root and in future lead to diminished plant shoot growth.

3.3 Metal content

3.3.1 Metal accumulation in the plant root

Heavy metals appear usually to accumulate more in the root than in the shoot of the plant. This general result was verified by the present experiment in the liquid growth medium (Figs. 6, 7, 8, 9). The electrical field might also influence the pattern of the metal uptake by the plants since the different elements may have different transport mechanisms in the plant. By applying an AC electrical field, the accumulation of Cd in the plant roots was enhanced, both under AC 10 Hz and AC 50 Hz compared to the control in the Cd contaminated nutrient (Fig. 6). The Cd accumulation in the plant root was 1,691 ± 218 mg/kg for AC 10 Hz, 1,314 ± 275 mg/kg for AC 50 Hz and 1,170 ± 7.4 mg/kg for control. The amount of cadmium in the plant root system was about five to six times higher than in the shoot (Fig. 6). Cadmium was added in the present experiment being as a target contaminant. However, copper, zinc and iron were existed as the micro-nutrients for the plant growth (Table 1). The electric field did not only provide an effect on the target contaminant uptake, but also influenced the mineral nutrient uptake in the plant. The Cu content of the roots was 10.1 ± 0.9, 10.5 ± 2.7 and 6.5 ± 1.8 mg/kg

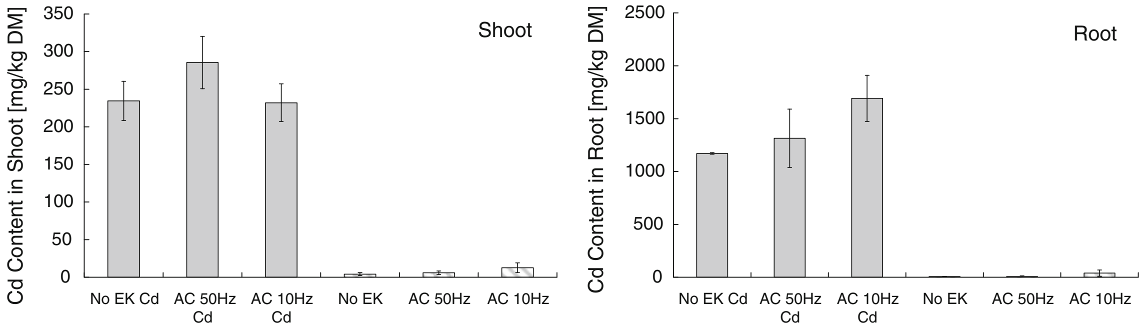

Fig. 6 Cadmium content in the plants (shoot and root) growing in non-contaminated and Cd contaminated solutions under three treatment conditions

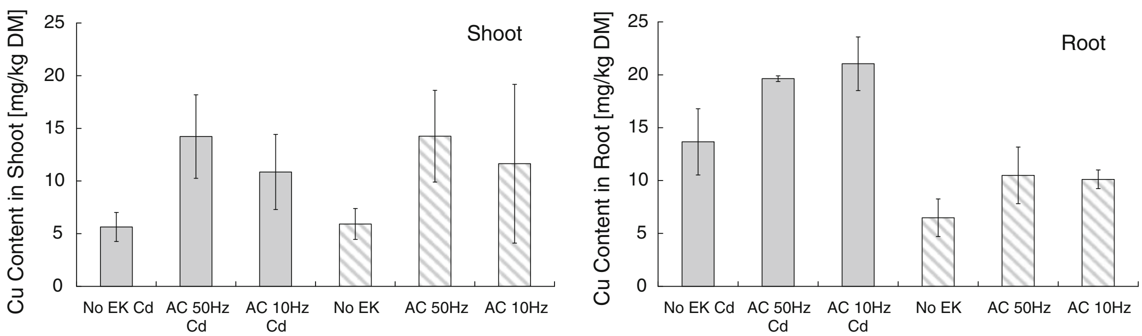

Fig. 7 Copper content in the plants (shoot and root) growing in non-contaminated and Cd contaminated solutions under three treatment conditions

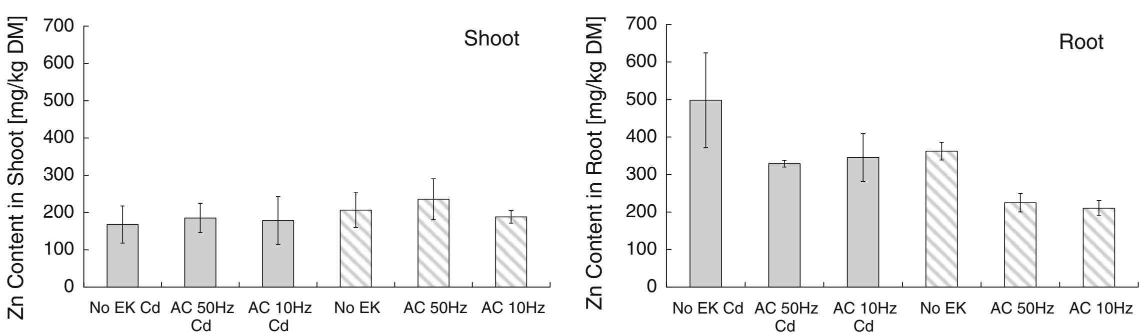

Fig. 8 Zinc content in the plants (shoot and root) growing in non-contaminated and Cd contaminated solutions under three treatment conditions

for the plant growing in the non-contaminated nutrient solution under the AC 10 Hz, the AC 50 Hz and the control, respectively. The Cu content in the plant roots was 21.0 $\pm$ 2.5, 19.6 $\pm$ 0.3 and 13.7 $\pm$ 3.1 mg/kg for AC 10 Hz, AC 50 Hz and the control in the Cd contaminated nutrient solution. With the presence of 5 mg/L of Cd in the growth medium, the root accumulation of Cu was increased (Fig. 7). This might be due to the change of the permeability of the root cell plasma membrane from the cadmium in the nutrient solution [6]. A similar situation was found for Zn content in the plant root (Fig. 8).

The mechanism of Cd uptake to the plant root is exchange absorption, diffusion and irreversible binding [22]. This is due to the large negative electric potential existing at the exterior surface of the root plasma membrane, provides enough energy to drive the uptake of Cd^{2+} [6]. However, the root cadmium is likely to be adsorbed on the root surface, and so it may be not available for translocation [23].

The accumulation of Cd in the plant root might diminish the capacity of the plant to accept other metal ions. The Cd^{2+} could be transported via a saturable cation transporter

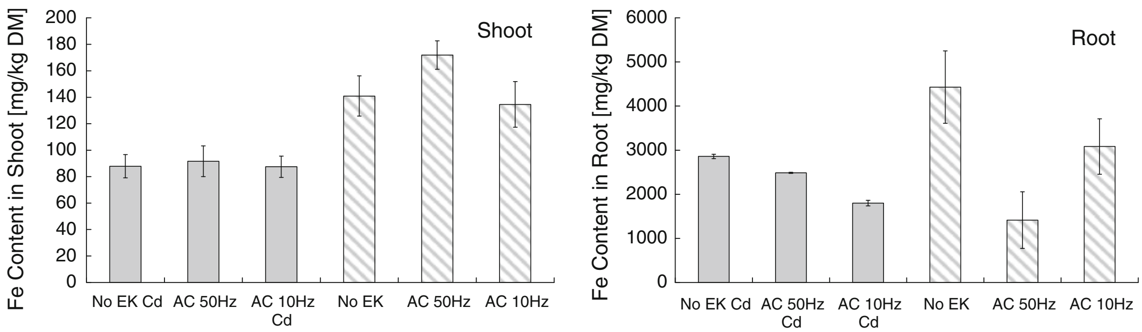

Fig. 9 Iron content in the plants (shoot and root) growing in non-contaminated and Cd contaminated solutions under three treatment conditions

in the plasma membrane and it could be diffused through a divalent cation membrane channel [24]. The pattern of iron accumulation in the plant root was opposite comparing to that for Cd accumulation in the plant root in the Cd contaminated growth medium (Figs. 6, 9). When a higher accumulation of Cd was found under the influence of AC field, reduced iron content was found at the plant roots. In addition, the Fe accumulation in the plant root was generally higher in the non-contaminated growth medium than in the Cd contaminated medium (Fig. 9). It was possible that the Cd^{2+} uptake dominated the cation channels for Fe^{2+} when cadmium was present in the growth medium. However, this speculative conclusion requires confirmation by the future research work.

Evidence of a metal competition effect was found between Cd and Zn (Figs. 6, 8). Higher accumulation of Cd in the plant root could eliminate the accumulation of Zn at the plant root, due to the similar mobility of Cd^{2+} and Zn^{2+}. They likely have the same carriers for transport. It was also noted that when excessive amounts of Zn are present, the Cd uptake in the plant could be eliminated and vice versa [25]. In the present experiment, under the influence of the electrical field, higher Cu and Cd accumulation was found at the plant root, thus the accumulation of Zn appeared to have been limited as a result of complex interactions between metals (Figs. 6, 7, 8).

3.3.2 Metal uptake in the plant shoot

In general, the AC electrical field significantly enhanced Cu uptake in the plant shoot, both at 10 and 50 Hz (Fig. 7). The shoot Cu content was two times higher for AC 10 Hz and 2.5 times higher for AC 50 Hz than for the control. The Cd uptake to the plant shoot was the highest under AC 50 Hz (Fig. 6), but the highest Cd accumulation at the plant root was found under AC 10 Hz. Cd prefers to form bonds with sulphhydryl ligand groups, but also binds to N and O ligand groups. Thus cysteine and other sulphhydryl containing compounds and various organic acids and other amino acids in the xylem sap could be important mechanisms for transporting Cd from roots to the shoots [6]. Comparing Cd content in the plant root and shoot, the highest overall translocation to the shoot was found in the plants treated with AC 50 Hz. The uptake of Fe and Zn in the plant shoot was similar with or without electrical field. However, comparing the Fe and Zn content in the shoot and root, a higher metal translocation factor from the root to the shoot was found under the influence of the electrical field (Figs. 8, 9).

3.3.3 Metal uptake per plant

Plant shoot metal uptake and plant biomass production are the most important factors for determining the efficiency of phytoremediation. In the present experiment, cadmium was added in the hydroponic system being as the target contaminant. Cadmium obtained in each plant was calculated by the following equation:

$$\begin{aligned}&\text{Metal content (mg/kg DM) in shoot}\\&\quad\times\ \text{Dry mass of the shoot (kg)}\\&\quad=\ \text{Metal uptake per plant mass}\end{aligned}$$

It can be seen in Fig. 10, the uptake of Cd per plant shoot dry mass was significantly enhanced by the application of the AC fields, both at 50 and 10 Hz in the Cd contaminated solution. The Cd uptake per plant part was about 90 and

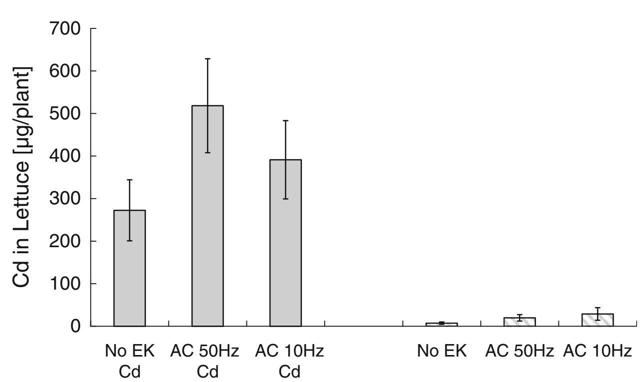

Fig. 10 Cadmium obtained per plant shoot mass

44% higher comparing to the plant without electrical treatment for AC 50 Hz and AC 10 Hz, respectively.

4 Conclusion

This laboratory scale experiment of electro-phytoremediation with a 10 Hz AC field and a 50 Hz AC field in a hydroponic growth medium demonstrated the possibility of using a low voltage AC electrical field to stimulate the lettuce plant growth, as well as to accelerate the uptake of metal ions. With the influence of an external electrical field in the plant growth medium, the accumulation of certain metal ions, such as Cu and Cd was increased from the bulk solution into the plant root system. Due to the selectivity of the plants to the absorption of metal ions, the higher accumulation of certain metal ion could also prohibit the capacity of the plant to accept other metal ions, causing a metal competition effect in the plants. The 50 Hz AC field showed potential not only in metal accumulation, but also in enhancing of metal ions from the plant root system to the plant shoot, which is one of the key factors in the application of phytoremediation.

The biomass production is another important factor for phytoremediation. To calculate the enhancement on biomass together with the enhancement on Cd content in the plant shoot under the AC treatments (10 and 50 Hz), the efficiency of phytoremediation was 90% higher under the influence of the AC 50 Hz electrical field and 44% higher under the influence of the AC 10 Hz electrical field.

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