

**COMPREHENSIVE STUDY OF ELECTROKINETIC-ASSISTED
PHYTOEXTRACTION OF METALS FROM MINE TAILINGS BY APPLYING
DIRECT AND ALTERNATE CURRENT**

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HIGHLIGHTS

Mobilization of metals was deeply assessed by exhaustive sampling of soil and water

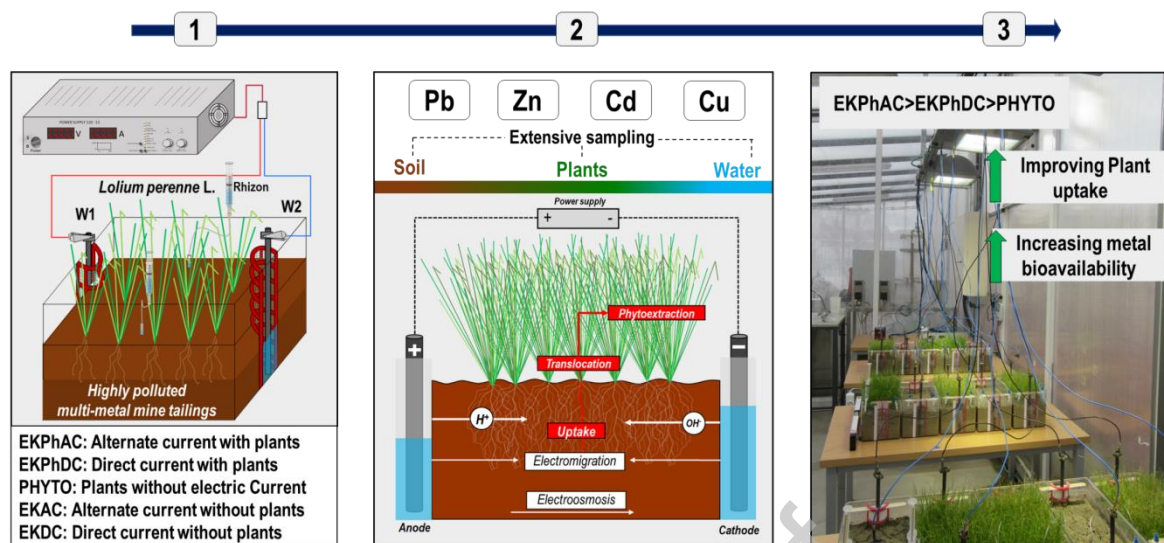
AC current was more effective in mobilizing metals despite not causing electroosmosis

Accumulation of metals in ryegrass tissues was significantly increased between 17-41%

BCR extraction showed that electric current increased Pb and Cu availability in tailings

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Graphical abstract

**ABSTRACT**

Electrokinetic-assisted phytoextraction (EKPH) involves the use of electric current to improve the mobilization of metals in soils and, therefore, their removal efficiency. It emerges as an interesting sustainable technology for the rehabilitation of abandoned mining areas. The goal of this research was to conduct a comprehensive study of this technique applied to real mine tailings from an abandoned Pb/Zn mine by using ryegrass as plant species and two types of electric current: direct with polarity reversal (DC-PR) and alternate (AC). The EKPH tests were conducted in specially designed plastic containers that allowed recording the main parameters of the electrokinetic process and sampling the different matrices studied, i.e. water, soil and plants. This intensive sampling program allowed detailed monitoring of the metal mobilization processes for the different treatments applied. The changes caused in metal mobility and physicochemical parameters of the tailings due to the application of electric current were reflected to a greater extent in the water samples than in solid ones. Accumulation

of Pb, Zn, Cd and Cu in ryegrass tissues increased significantly by 41%, 17%, 34% and 32% when applying 1 V cm^{-1} of AC current, with respect to the results of phytoextraction without electric current. The application of DC-PR current did not lead, in general, to statistically significant increases in the plant metal uptake. These findings were adequately correlated with the changes found in the metal concentrations of soil pore water and, to a lesser extent, with those observed from the BCR sequential extraction applied to tailings samples.

Keywords: metals, electrokinetic-assisted phytoextraction, mine tailings, ryegrass, AC current, DC current

1. INTRODUCTION

The contamination of water sources and soils by industrial and mining activities has become a global concern due to the associated environmental and health human risk [1,2]. Among the different pollutants, metals are particularly hazardous because they are recalcitrant substances that can be accumulated along the food chain [3]. Some sustainable technologies have been implemented as a mitigation measure to reduce the negative impact of these pollutants. Phytoremediation is currently considered an economical and environmentally friendly option for the treatment of soils contaminated with metals [4]. Metals are removed from soil or other solid matrices through the absorption and accumulation in plant tissues, ideally in the aerial ones; this process is called phytoextraction. One of the most recognized drawbacks of phytoextraction is its relatively reduced effectiveness due to the usual low availability of metals in polluted soils, especially if the contamination has been aged, such as in abandoned mining areas; it often makes the time required to carry out soil decontamination too long. The most

widely used way to increase the availability of metals is the addition of chelating chemicals, such as ethylenediaminetetraacetic acid (EDTA), ethylenediamine-N,N'-disuccinic acid (EDDS) or diethylenetriamine pentaacetate (DTPA), which allows generate more bioavailable metal complexes capable of diffusing in the soil and plant tissues in a more efficient way [5]. However, metal leaching and the subsequent groundwater contamination can potentially be increased by this type of treatment [6]. To avoid this disadvantage, the combination of electrokinetic remediation and phytoremediation has been studied in the last decade [7,8]. When direct electric current (DC) is applied to a soil, the electrolysis of water, generating acid and basic fronts at the anode and cathode, respectively, along with electrokinetic phenomena, i.e. mainly electroosmosis and electromigration, increase the mobility and transport of metal ions through the soil [9–12]. Therefore, electrokinetic-assisted phytoremediation (EKPH) emerges as an interesting alternative to chelating agents to increase metal availability and shorten remediation times.

Although EKPH was initially conceived to take advantage of the mobilization and transport of metal ions caused by the application of DC current between the electrodes, it has been shown that this type of unidirectional current leads to the creation of two fronts, acid and basic (at anode and cathode, respectively). It causes detrimental effects on plant growth [13,14] and metal precipitation at the cathode [15,16]. Two strategies have been used by some researchers to avoid this problem: (i) periodically inverting the polarity, which is called 'polarity reversal' mode (DC-PR), and (ii) applying alternating current (AC). It has been shown that the DC-PR mode does not cause significant pH changes and leads to the accumulation of metals in the zone between the electrodes due to the crossing between the acid and basic fronts [17,18]. Likewise, some less numerous

studies have included the use of AC current in EKPH, demonstrating that metal mobilization can be achieved without affecting plant growth [19–21].

Despite of the advances made in the last ten years in the field of EKPH, an excessive number of studies are still not available (less than 40 bibliographical references with very few examples from real mine soils) and some important aspects of the technique have not been adequately elucidated, as noted in a recent review by us [8]. We suggested that comparative experiments using different types of electric field application (DC, AC or DC-PR) should be carried out under the same experimental conditions in order to clarify their performance on plant metal uptake and biomass development. In addition, the existence or not of a continuous electroosmotic flow in tests with AC current has not been clearly established. Lastly, another issue that has been little studied is how electricity affects the fractionation of metals in the soil and their availability; to date, experimental techniques such as sequential extraction methods (to assess the geochemical partitioning of metals in soil) and soil pore water sampling and analysis (using Rhizon samplers) have rarely been used in the EKPH field.

The present study aims to address some of the challenges that currently exist in the field of EKPH research, including: (i) to conduct the experiments using real mine tailings with high concentration of several metals, (ii) to assess the differences on using AC or DC current, and (iii) to carry out a complete sampling program that allows monitoring the mobilization patterns of metals in both the solid and aqueous phases of the soil. Thus, an electrokinetic-assisted phytoremediation test was carried out using *Lolium perenne* L. (English or perennial ryegrass) as plant species, tailings from an abandoned

Pb/Zn mine as a growth substrate, plastic containers specially designed to visualize the height of the soil water in the electrode wells and two types of electric field, i.e. alternate current and direct current with polarity reversal.

2. MATERIALS AND METHODS

2.1 Mine tailings collection and characterization

Samples of highly polluted multi-metal mine tailings were taken from the abandoned San Quintín mine, located in south-central Spain (UTM coordinates: 389484, 4297643). It was mined from the end of the 19th century to the first half of the 20th century for the extraction of sphalerite (ZnS) and galena (PbS) [22]. After field sampling, the tailings (200 kg) were air-dried under room temperature and sieved to 2 mm. The growth substrate (also referred to as 'soil' in the text) used for the experiment consisted of a mixture of mine tailings and washed sand 75:25 w:w. The total metal concentrations of the resulting growth substrate were 3832, 2904, 94.0 and 38.7 mg kg⁻¹ of Pb, Zn, Cu and Cd, respectively. pH and electrical conductivity were 6.8 and 0.66 mS cm⁻¹, respectively. Other physicochemical properties of this substrate are shown in Table SM1 in Supplementary Materials.

2.2 Experimental set-up and design

Five different treatments were used in this experiment: (i) ryegrass under alternate current, EKPhAC; (ii) ryegrass under direct current (with polarity reversal), EKPhDC;

(iii) ryegrass without application of electric current, PHYTO; (iv) unseeded substrate and alternate current, EKAC, and (v) unseeded substrate and direct current (with polarity reversal), EKDC. The experimental test was carried out using a randomized block design with three replicates.

FIGURE 1

Clear plastic rectangular containers (27 cm long, 17 cm wide, 18 cm high) were filled with 7 kg of growth substrate (Figure 1); it was slightly compacted until reached 10 cm in height. The field capacity of the substrate was around 2000 mL of water per container, equivalent to 28.5% by weight. Semi-cylindrical wells with nylon mesh walls (120 mesh, 5 cm diameter) were placed at both ends of the container allowing free flow of water through the substrate and visible water surfaces in both wells. Graphite carbon electrodes (1cm-diameter and 25 cm-height) were plunged vertically into these wells to the bottom. Additionally, six containers were planted, 3 with the polluted substrate and 3 with uncontaminated agricultural soil, with *Lolium perenne* L. to evaluate the phytotoxicity effects of the polluted substrate and take samples of plant tissues and substrate before starting the application of electric current; these containers were sacrificed the day the electric current was applied (44 days after ryegrass sowing).

2.3 Methodology of plant growth and electric current application

English or perennial ryegrass (*Lolium perenne* L.) was sown in the plastic containers (PHYTO, EKPhAC and EKPhDC treatments and that of the agricultural soil) using a

dose of 100 g of seeds per m². The experimental trial was carried out for 58 days in a greenhouse equipped with temperature and artificial light control. The culture conditions were 22°-29°C/11-14°C temperature day/night, 12 light hours/day (from 8:00 a.m. to 6:00 p.m.) with the support of artificial light (Philips Master HPI-T Plus Metal Halide Lamps; 400 w). All containers were fertilized with a solution of N, P and K after the sowing stage. Daily irrigation was conducted (after disconnecting the electric current and proceeding to the sampling and measurement of experimental parameters) in order to maintain the field capacity of the substrate. It was done by adding water to the wells to compensate for the loss of water by evaporation (EKAC and EKDC treatments) or evapotranspiration (EKPhDC, EKPhAC and PHYTO treatments); it led to a water height of approximately 8 cm in both electrode wells.

44 days after sowing, a voltage gradient of 1 V cm⁻¹ was applied, 8 hours a day (from 10:00 a.m. to 6:00 p.m.) for 14 days, to the EKAC, EKDC, EKPhAC and EKPhDC treatments (feeding DC by Delta Elektronica S.V., model SM120-13, The Netherlands; AC power supply by Polylux, model TRAF0 QB200, Spain). The electrode that started to function as anode was indicated as W₁ while the other was indicated as W₂ (cathode). The polarity of the DC current was changed after 4 h each day (at 2:00 p.m.).

2.4 Sampling and chemical analysis

pH, electrical conductivity (EC), temperature and height of the water level in both electrode wells were monitored at three different times of each day: (i) before switching on the electric current ($t = 0$ h), (ii) before changing the polarity of the electrodes in the

treatments with DC current ($t = 4$ h), and (iii) before disconnecting the electric current ($t = 8$ h). The intensity of the electric current was measured every day at $t = 0$ and 8 h for the AC current treatments and at $t = 0, 4$ and 8 h for those with DC current. Soil pore water samples were taken daily at $t = 8$ h by means of two Rhizon samplers (both samples were combined in only one) from the central section of the containers; they were analyzed for pH and EC. Total concentration of Pb, Zn, Cd and Cu was analyzed in the water samples taken at $t = 8$ h in both electrode wells and from the bulk soil (five samplings in days 46, 49, 52, 55 and 58). Once the entire experiment was finished (after 58 days from sowing and 14 from the application of electric current) soil samples were collected from central section of each container; they were air-dried at room temperature for 24 h and ground in a ball mill prior to digestion. The total concentrations and the geochemical partitioning of Pb, Zn, Cd and Cu in soil samples were analyzed; the activity of dehydrogenase enzyme was also assessed in soil samples that had been stored at -17°C . The complete biomass of the ryegrass grown in the containers was collected at the end of the experiment; it was split in shoot and root biomass, being the roots carefully separated from the soil and recovering them almost in their entirety. Next, the plant samples were washed with deionized water, dried in an oven at 80°C for 24 h, and ground in an ultra-centrifugal mill.

Electrical conductivity and pH were measured using a soil-water ratio of 1:5 (w:v). Digestion of soil and plant samples was conducted according to the EPA 3051A method; in brief, 0.5 g of ground sample was digested using a mixture of 9 ml of concentrated nitric acid and 3 ml of concentrated hydrochloric acid in a microwave digester. For quality assurance, one blank and one sample of a certified reference material (SQC001 from Sigma-Aldrich, for soils, and ERM-CD281 from European

Reference Materials, for plant samples) were added to every 10 samples. The geochemical partitioning of metals in the soil was determined using the modified BCR sequential extraction method that allowed obtaining four geochemical fractions of metals: (i) exchangeable, water and acid soluble, obtained by extraction with acetic acid 0.11 M (F1); (ii) reducible or bound to Fe-Mn oxides, resulting from extraction with 0.5 M hydroxylamine hydrochloride at pH 1.5 (F2); (iii) oxidizable or bound to organic matter and sulfides, obtained by reaction with 8.8 M H₂O₂ followed by an extraction with 1.0 M ammonium acetate at pH 2 (F3); and (iv) residual or bound to non-silicate minerals, obtained using the previously mentioned 3051A EPA method (F4) [23,24]. To ensure quality, two blanks and one certified reference material (BCR-701 from European Reference Materials) were added to each 9 samples. All extracts and liquid samples were analyzed for metal concentration using an ICP-MS spectrometer (model Thermo iCAP TQ from ThermoFisher Scientific, Waltham, MA USA). Soil dehydrogenase enzyme was analyzed by reducing triphenyl-tetrazolium chloride to triphenyl-formazan and measuring the obtained solution photometrically at 546 nm [25,26].

Phytoextraction potential was evaluated using three parameters widely used in phytoremediation such as the bioconcentration factor (BCF), the accumulation factor (AF) and the translocation factor (TF). These were calculated using the following equations [27,28]:

$$BCF = \frac{\text{metal concentration in roots}}{\text{metal concentration in soil}} \quad (1)$$

$$AF = \frac{\text{metal concentration in shoots}}{\text{metal concentration in soil}} \quad (2)$$

$$TF = \frac{\text{metal concentration in shoots}}{\text{metal concentration in roots}} \quad (3)$$

2.5 Statistical analysis

The Statistix 10 package (Analytical software, USA) was used to apply the analysis of variance (ANOVA) to each parameter measured according to the experimental design (randomized complete block). The mean values of the different parameters were compared using Fisher's Least Significant Difference (LSD) test at $p \leq 0.05$. The temporal profile of the metal concentrations in soil pore water for the different treatments was analyzed by using two way-ANOVA and the LSD test and calculating main effects and interactions (between the type of treatment and the days of the experiment) according to Gómez and Gómez [29].

3. RESULTS AND DISCUSSION

3.1 Physicochemical parameters of water in electrode wells and bulk soil pores

FIGURE 2

As described in Section 2.4, the distinctive design of the containers used in the EKPH test allowed the height reached by soil water in both electrode wells (W1 and W2) to be recorded every day in three separate times. In general, the height of the water in both

wells decreased throughout the day in all treatments due to plant evapotranspiration (in treatments with plants) and evaporation from the soil (in unseeded treatments). These water losses cannot be attributed to ohmic heating because they were similar in all treatments, being mainly influenced by the ambient temperature of the greenhouse. Likewise, since the water was replenished every day, a more or less constant pattern of variation was observed throughout the entire experiment. In Figure 2 it can be seen that the difference between the height reached by the water in both electrode wells (mean values for all replicates and 14 days of experiment) remained constant at very low values (a few millimeters) throughout the day for the treatments with no electric field application and those with AC current application; however, as expected, height differences of several centimeters (2.3-3 cm) were detected after 4 hours of DC current application (EKDC and EKPhDC treatments). This clearly illustrates the transport of water by electroosmosis from the anode to the cathode when DC current is applied to the soil [19]. On the contrary, from our results, it can be said that the electroosmotic flux was not established under AC current. When the polarity of DC current was changed (W1: cathode, W2: anode), the electroosmotic flow direction was reversed (keeping the anode-cathode direction). However, the final height difference after the second interval of 4 hours of current application was quite lower (< 0.5 cm) than that of the first 4 hours interval (Figure 2). Several reasons could contribute to this fact: (i) the different initial water levels in the electrode wells (the initial height difference was not zero for $t = 4$ h) along with the water losses during the test; (ii) the time required for the flow reversal mechanisms to begin to develop; and (iii) the high EC of soil pore water after 4 h of current application that could increase the zeta potential of soil particles, thus reducing the electroosmotic flux [15]. Lastly, it can be seen in Figure 2 that the highest differences in the height of the water level in electrode wells were found for the

treatment with DC current and plants (EKPhDC series); it can be attributed not only to the electroosmotic flow but also to the increase in the hydraulic flow through the soil pores induced by plant roots; in fact, it was observed that the ryegrass root system heavily colonized all the soil volume available in the growth containers.

FIGURE 3

Figure 3 shows the daily variation of pH and EC (mean values for all replicates and 14 days of experiment) in the water from both electrode wells (Figures 3a and 3b, respectively) along with the pH and EC values of the soil pore water collected (by means of Rhizon samplers) every day after turning off the electric current (Figure 3c). Firstly, it can be observed that the average pH values of the water remained practically constant (in the range 7.3-7.8) in the treatments corresponding to the application of AC current (EKAC, EKPhAC) and without electricity (PHYTO) (Figure 3a). However, as expected, for the treatments that applied DC current, the electrolysis of the water caused the pH to change rapidly to extreme acidic (1.85 for anode) and basic (12.15 for cathode) values [1,32]; moreover, these extreme values were also reached to a lesser extent, but in the opposite wells, when the current polarity was reversed (Figure 3a, $t = 8$ h). It is also of note that the pH of the water in the electrode wells returned to its initial value every day after 16 hours without application of electric field (Figure 3a, $t = 0$ h). The pH of the interstitial soil water samples taken daily after the application of electric current in the central section of the plastic containers remained at similar neutral values for all treatments (Figure 3c); it means that the polarity reversal mode in DC treatment (along with probably some tailings buffering capacity) was efficient in avoiding

extreme pH values in the bulk soil that could affect the growth of the plants. This fact also explains why the pH of the water in the electrode wells returned to neutral values every day after the application of DC.

The behavior of the EC was quite different in treatments with DC current application and the rest of the treatments. Regarding the water in the electrode wells, EC remained approximately constant at relatively low values ($0.36\text{-}0.37\text{ mS cm}^{-1}$) for all treatments except the one corresponding to the application of DC current. In the latter case, the EC of the water greatly increased at the anode (to 8.41 mS cm^{-1}) and cathode (4.39 mS cm^{-1}) after applying current for 4 hours; it was due to the generation of H^+ and OH^- ions from the electrolysis of water at the anode and cathode, respectively [9]. The higher EC of the water at the anode can be explained by the higher ionic conductivity of the H^+ ions in relation to OH^- ions ($349.8\text{ vs }198.6\text{ S cm}^2\text{ mol}^{-1}$); it is in agreement with previous findings in EKPH tests using DC current [13]. Nevertheless, other ions in the soil, such as sulphate, chloride and cations of alkali elements, alkali earth elements, aluminum, iron and heavy metals, could also be transported to the anode or cathode, depending on their electric charge, by electromigration and contribute to the total EC of the water in electrode wells [15]. When the polarity of the DC current was reversed ($t = 8\text{ h}$, Figure 3b), the EC of the water in both electrode wells decreased significantly (1.65 and 1.55 mS cm^{-1} , for the anode and cathode, respectively). It can be attributed, on the one hand, to the partial neutralization of hydrogen and hydroxide ions in the electrode wells; on the other hand, hydrogen ions can also decrease due to the dissolution of the metal hydroxides that could have previously precipitated in the soil area in the vicinity of the cathode [12]. It would also agree with the aforementioned less extreme pH values

achieved in the electrode wells when the current polarity was reversed ($t = 8$ h, Figure 3a).

Regarding EC of the water samples taken from the bulk soil in the middle section of the containers (Figure 3c), it can be observed that this parameter was in the range 6.32-10.95 mS cm^{-1} for all the tested treatments; these high EC values cannot be explained in terms of the H^+ and OH^- ions due to the approximately neutral values found for all the experimental series (Figure 3c). Then, since the soil conductivity depends on the concentration and mobility of the ions present [11], the EC values of the soil pore water are related to the mobilization of soil ions achieved in the different treatments tested here. The order of EC was $\text{EKPhAC} > \text{EKPhDC} > \text{PHYTO} \approx \text{EKDC} > \text{EKAC}$, showing the synergistic effect of plant roots and electricity to increase the mobility of metal and salt ions in the mine tailings substrate. The subsequent discussion on metal concentrations in soil pore water and soil samples will help us establish whether the observed increase in EC is due to the mobilization of metals coming from mining activities or due to other cations or anions present (e.g. sulphates). The essential role of plants in increasing conductivity can be explained by the retention of salt ions in the vicinity of the roots together with the secretion of plant metabolites (root exudates), such as organic acids [34,35]. Among other similar cases, the secretion of tartaric acid by ryegrass in response to Pb stress has also been previously reported [36].

3.2 Behavior of physicochemical parameters of bulk soil

TABLE 1

Table 1 shows the mean values of pH and electrical conductivity (EC) of the soil before and after the applied treatments. The soil pH did not vary significantly from its initial value and was similar for the different treatments. It means that both the application of AC and DC current (with polarity reversal) avoided significant changes in soil pH that could affect soil fertility. It is in agreement with what found for soil pore water (Section 3.1) and with the findings of other authors using AC current [27] or DC current with polarity reversal [33]. The neutralization of the acid and basic fronts in the case of DC current along with the buffering capacity of the soil can be suggested as the main reasons for this behavior [4,7,33]. The electrical conductivity of the soil was significantly increased from its initial value but not significant differences between treatments were found. So, in the same way as described above for the soil pore water (Section 3.1), it can be said that the mobilization of soil ions by the electric field is capable of increasing the total EC of the soil (more than twice the initial value) although the changes were not so important to distinguish between different treatments. In the case of the treatment without electric field application (PHYTO treatment), the rise of soil EC can be attributed to the release of root exudates by plants.

The electric intensity did not vary significantly throughout the day in the treatments that applied AC current (Figure SM1 in Supplementary Materials). On the contrary, for DC current treatments, the electrical intensity increased a lot from the beginning of the application of the current until the first 4 hours, and then, after 8 h of current application, it decreased to slightly lower values than the initial ones ($t = 0$ h); it is the same trend previously described for the EC of the water sampled in electrode wells

(Figure 3b). The electric current measured in electrokinetic remediation experiments has previously been shown to be related to ions flowing through the soil, moisture, porosity and electrochemical reactions occurring on the electrode surfaces [30]. Considering a similar magnitude for the electrochemical reactions in both electrodes, the variation of the ionic concentration of water in the electrode wells would cause the electric current changes observed in the treatments using DC current. In addition, the treatments with plants showed electric intensity values higher than those corresponding to the respective treatments without plants (Figure SM1); in fact, plants roots can increase soil porosity by increasing hydraulic flow through the soil and favoring ion transport [37].

3.3 Metal concentrations in water from soil pores and electrode wells

FIGURE 4

Figure 4 shows the concentration of Pb and Zn in the water samples taken from the electrode wells (W1 and W2) and the bulk soil on different days after the daily application of electric current (similar figures have been included for Cd and Cu as Supplementary Materials in Figure SM2). Regarding the electrode wells, it can be seen that the metal concentrations were exceptionally low or negligible at the cathode (W1) for all the treatments, while they reached statistically significantly higher values at the anode (W2) for the treatments applying DC current; a similar behavior was also found for Cu and Cd (Figure SM2). First, it can be pointed out that only direct current is capable of establishing an electroosmotic flux that can transport metals and other ions

between the electrode wells. However, since the flow of metals is from anode to cathode (both by electroosmosis and electromigration), the fact that higher concentrations of metals were found at the anode (W2) means that they, probably, came mainly from the solubilization of metal hydroxides in the soil zone closed to the anode well due to the acidic pH generated. Those hydroxides would have been formed when metals flow towards the cathode and encounter an extremely basic pH in the soil area near the cathode; it would avoid a high concentration of metals in the electrode well that functioned as cathode when the samples were taken (W1). It agrees with what was reported by Yuan et al. [16] for the electrokinetic-assisted phytoremediation of a multi-metal polluted soil using reverse-polarity DC current. In addition, it is also consistent with the observations on the pH and EC values of the water in electrode wells previously described in Section 3.1.

As for the concentrations of metal in the water extracted from the bulk soil by the Rhizon samplers, in Figure 4 some differences between the concentration profiles of Pb and Zn can be seen. While mean Pb concentrations in soil pore water did not change significantly for the different treatments, Zn concentrations did (see also Supplementary Material, Table SM2). Statistically significant differences were found between the average values of Zn concentration (means of the five samplings) corresponding to the treatments with plants (110.9, 99.3 and 64.0 mg kg⁻¹ for EKPhAC, EKPhDC and PHYTO series, respectively) and those without plants (12.1 and 17.7 mg kg⁻¹ for EKAC and EKDC series, respectively) (Table SM2); the mean Zn concentration of the EKPhAC treatment was also significantly higher than that of the PHYTO. The behavior of Cu and Cd was similar to that of Zn (Figure SM2 and Table SM2). The highest Pb concentrations were also found for the EKPhAC and EKPhDC series (average values of

0.79 and 0.86 mg kg⁻¹, respectively; Table SM2) but the differences with the other treatments were not significant as in the case of Zn (and Cd and Cu); it could be due to the lower solubility of Pb soil species and salts (such as lead sulphate) relative to the other metals studied [38]. These facts are substantially similar to those observed for the EC of the soil pore water (Section 3.1) and allow to clarify the relevant role of the released metals in the observed increase of the EC. It also seems clear that both types of electric current (AC and DC) improved the metal mobilization effect performed by ryegrass roots and did so in a similar way; therefore, it can be said that electroosmotic flux generated by DC current was not more effective for the mobilization of metals than other mechanisms developed by AC current. On the other hand, concentration of soluble metals increased as the experiment progressed, although in the last days it remained constant or decreased depending on the metal considered (Figures 4 and SM2, Table SM2); it would indicate a certain limitation in the capacity to mobilize metals due to the release of the most labile fractions in the first days of application of the electric current. Additionally, significant interaction effects were found between the experimental factors 'treatment type' and 'experiment days' for Zn and Cu but not for Pb and Cd (Table SM2). It means that the differences between the mobilization patterns of Zn and Cd found for the combined treatments were more important as the experiment progressed; it could be related to the greater availability of these metals in the tailings used. On the contrary, the metal concentrations in the anode well (W2) did not follow a clear trend throughout the experimentation time; the different volume of water in the electrode wells, depending on the ambient temperature and sunlight, could condition the concentration of metals to a certain extent.

3.4 Changes in soil metal fractionation

FIGURE 5

The BCR sequential extraction method was carried out in tailings samples taken before and after the treatments in order to detect changes in the geochemical fractionation of the metals; the results are shown in Figure 5. It can be seen that the exchangeable fraction (F1) was the most abundant for Zn, Cd and Cu, while Pb was mainly associated with Fe-Mn oxides (F2 fraction). Cu also showed high concentrations for F2 and F4 fractions, like Pb. Therefore, it can be said that availability of metals in tailings followed the order $Cd \approx Zn > Cu \approx Pb$. Likewise, the oxidizable fraction (F3) was the lowest for all metals, probably due to the low organic content of the tailings (<1% of organic carbon, Table SM1). It agrees with what was reported by other authors [39,40] and with our previous findings for mine tailings from the San Quintín mining area [24].

The application of electric current did not lead to significant changes, with respect to the initial mine tailings, in the F1 fraction of Zn, Cd and Cu. However, Pb was significantly increased in the F1 fraction for the EKAC treatment and, to a lesser extent, for the EKDC and EKPhDC ones. Zn and Cd concentrations of the F2 fraction increased significantly for all applied treatments compared to the initial soil. Finally, despite the low concentration of metals in the F3 fraction, it increased for Zn and decreased significantly for Pb after the application of all treatments. The F4 residual fraction did not change significantly for any treatment. The only significant difference between electrokinetic treatments and the phytoextraction one (PHYTO series) was found for the

concentration of Pb in the F1 fraction, which was significantly higher for the EKAC and EKDC treatments.

The results described here for the speciation of metals showed some changes, very little marked in some cases, after the treatment of the mine tailings both with an electric field and/or plants; these changes implied increases in the non-residual metallic fractions, which are the metals that could be taken up by plant roots. Cang et al. [7] reported the increase in bioavailable soil concentrations of Cd, Zn and Cu in the EKPH of an industrial soil by Indian mustard using unidirectional DC current. Other authors have shown that it is possible to increase the concentration of bioavailable Cd by applying AC current [41] and DC current with polarity reversal [42,43]. However, those changes in metal availability were not always correlated with the accumulation of metals in plants [8]. On the other hand, the similar effectiveness of ryegrass in changing metal speciation compared to treatments using electric current can be explained by taking into account that, on the one hand, plant roots release organic acids that can increase the availability of metals in the rhizosphere environment as response to pollution stress (such as that caused by metals) [44] and, on the other hand, the ryegrass plants could have been releasing exudates during the first 44 days before starting the application of electric current, which lasted only 14 days. Therefore, the longer duration of the PHYTO treatment could significantly condition the fractionation of the metals and must be carefully considered in relation to the other treatments. In addition, the uptake of available metals by the plants can also condition their geochemical fractionation after the experiment; thus, the changes were more evident for the treatments without plants (EKDC and EKAC), unlike those described for the aqueous phase of the soil (Section 3.4) where the changes in metal availability were faster and less conditioned by the

absorption of metals by plants. Finally, it should also be mentioned that since no measurable dehydrogenase activity was detected in all applied treatments, any role of soil microorganisms in metal mobilization should be ruled out.

3.5 Plant biomass and metal uptake

The dry weight of ryegrass did not show statistically significant differences between the different treatments applied in this study; likewise, the shoot biomass was approximately twice that of the roots (Figure SM3 in Supplementary Materials). However, other authors have reported both negative and positive effects of the application of electric current on plant biomass [8]. O'Connor et al. [1] and Zhou et al. [6] found no adverse effects on ryegrass growth in an EKPH test using DC current; however, we reported that ryegrass biomass decreased by 9 to 32% when DC current with reverse polarity was applied [14]. Likewise, most EKPH studies using AC current have reported a beneficial effect on plant growth [5,17,19,41]; it would be mainly due to the periodic hyperpolarization and depolarization of cell membranes which causes an increase in the entry of ions into the root cells [19]. In our study, the effect of electric current was insignificant, probably due to the short application time (14 days out of a total of 58 days) and, to a higher extent, because plant growth was dramatically conditioned by the toxicity of the metals. In fact, ryegrass showed signs of chlorosis and early wilting in all treatments and, furthermore, ryegrass grown on uncontaminated agricultural soil under the same conditions as that grown on mine tailings substrate reached approximately 3-times higher plant biomass (Figure SM4 in Supplementary Materials).

TABLE 2

The contents of Pb, Zn, Cd and Cu accumulated in ryegrass tissues under different treatments are shown in Table 2. Zn was by far the metal that reached the highest rate of accumulation in roots and shoots (6703-7890 and 1122-1464 mg kg⁻¹, respectively); it was followed by Pb, which accumulated mainly in roots (2339-3388 mg kg⁻¹). Cd and Pb reached similar concentrations in ryegrass shoots, i.e. 59.89-78.63 and 58.83-72.30 mg kg⁻¹, respectively. Lastly, Cu reached concentrations in roots similar to those of Cd, but its concentration in shoots was the lowest of the four metals studied. Regardless of the treatment, the order of total metal accumulation in plants was Zn > Pb > Cd ≈ Cu. Compared to previous literature [45–47], it can be said that metal concentrations obtained in plants roots were extremely high, which is indicative of a high availability of metals in the mine tailings used as growth substrate. The bioconcentration factors (BCF) were especially high for Cd and Cu (19.31-26.02 and 9.59-11.73, respectively) with more moderate values for Zn (2.40-2.77) and low for Pb (0.58-0.89). It agrees with the relatively high metal concentrations found for the F1 fraction of Zn, Cd and Cu from the BCR sequential extraction (Section 3.4). Furthermore, this fact clearly highlights that metal uptake depends on the amount of metal available in the vicinity of the roots, which is a function of its total concentration and its availability.

The shoot metal concentrations of Zn, Pb and Cd also reached levels that can be considered quite high [45]; in fact, the Zn concentrations in shoots were higher than 0.1% of their dry weight. Such concentrations are considered toxic to plants [38]; it would explain the symptoms of toxicity and the loss of biomass observed in this

experiment and described in the previous section. Therefore, the low values obtained for the translocation factors (TF, Table 2) should be taken with care, since they do not indicate that ryegrass is not capable of translocating metals, but rather that the translocation rate is much slower than the root absorption rate. Nevertheless, some plant resistance to contaminant translocation should not be ruled out to avoid toxicity [48]. Accumulation factor (AF) reached values between 1.47 and 1.92 for Cd, while it was lesser than 1 for Pb, Zn and Cu (Table 2). The low values obtained here for AF and TF (< 1) indicate that ryegrass would not be a good candidate for phytoextraction but rather for phytostabilization [46,49]; nevertheless, it should not be forgotten that the true objective of this research was to assess whether electric current is capable of enhancing the accumulation of metals in ryegrass tissues.

Regarding the accumulation of metals for the different treatments, it can be seen in Table 2 that, in general, higher plant metal concentrations were obtained for the treatments with the application of electric current compared to those of phytoextraction treatment. However, the results should be discussed in more detail for the different metals studied, type of electric field and plant tissues. Given the relatively high standard deviation values, none of metals showed significant differences between treatments for shoot concentrations; however, the differences were more evident for roots, where translocation mechanisms are not involved. The concentration of Pb in roots showed significant differences between the different treatments, being the order of accumulation $EKPhAC > EKPhDC > PHYTO$. The root concentrations of Cu and Cd found in the treatment with AC current were significantly higher than those of DC current and phytoextraction treatments, while no significant differences were found for Zn root concentrations. In general, the plant concentrations of Pb, Cu and Cd reached in the

treatment using AC current were significantly higher than those of phytoextraction; indeed, plant accumulation of Pb, Zn, Cd and Cu in ryegrass was increased in 41%, 17%, 34% and 32%, respectively. The application of DC current caused significant increases with respect to phytoextraction only for the concentration of Pb in roots. This means that the changes caused in the availability of metals in the mine tailings due to the application of the electric field affected more importantly, in terms of plant uptake, non-essential metals, i.e. Pb and Cd; however, the accumulation of Zn, which is an essential plant micronutrient and initially highly available, in ryegrass was not significantly enhanced. The values of TF, AF and BCF were not significantly affected by the application of electric current.

Some other authors previously reported the improvement of plant metal concentrations when AC current was applied [17,19,21]; in other cases, a higher metal uptake was mainly due to the improvement in plant biomass caused the application of AC current [43]. Reversal polarity DC current application has also been shown to increase metal accumulation, although it was dependent on plant species and the applied voltage gradient [19,43]. Moreover, some studies have found, in agreement with the results shown here, that AC current led to higher plant metal uptake than DC current with polarity reversal under the same experimental conditions [17,19,43,50]. Due to the different ease of metal transport from roots to shoots, it has been shown that root metal concentrations are a better indicator of metal availability in soils than shoot concentrations [24]; our results show that uptake of metals by the roots was enhanced when the concentration of metals in the rhizosphere increased. In this research, it was found that metals were mobilized to a similar extent in mine tailings by both AC and DC current application (Section 3.3); therefore, the higher concentrations of metals

accumulated in plants achieved with AC current can be attributed to the fact that this type of electric field is capable of causing changes in the absorption mechanisms of the root cells membranes and improving the metabolism of the plants [19]. In addition, it could be hypothesized that the improvement of the metabolic activity in the soil-root interface caused by alternating current could increase the release of root exudates that would improve the mobilization of metals. However, the enhancement obtained in this work for the accumulation of metals in the EKPH experimental series with respect to those of the phytoextraction series was relatively low; it can be attributed to two reasons: (i) the high initial availability of metals in the mine tailings and (ii) the relatively short period of application of the electric field compared to the total growth time of the plants in the contaminated soil (14 vs 58 days).

4. CONCLUSIONS

In this work, the combination of phytoextraction and the application of an electric field (alternating current and direct current with polarity inversion) for the removal of metals from mine tailings highly contaminated with Pb, Zn, Cu, and Cd was deeply studied. An extensive sampling and collection of measurements of the physicochemical parameters of the soil, water and plants have allowed establishing metal mobilization mechanisms and their extent depending on the type of electrical current. The main conclusions drawn from this research are the following:

- The application of DC current led to low pH values in the anode well and high ones in the cathode; EC reached higher values at the anode. For the treatments with the application of AC current and without electricity, the pH and EC values remained the same in the anode and cathode wells.
- The analysis of the pH and EC values measured in the soil pore water samples better reflected the changes in the physicochemical properties of the soil matrix caused by the electric field and the plants than the measurements in solid soil samples.
- The highest concentrations of Pb, Zn, Cu and Cd in the interstitial water of the soil were found for the treatments with electric field and plants, thus demonstrating the synergistic effect of both agents in the mobilization and transport of metals through the soil.
- The metal mobilization mechanisms developed by combined treatments based on alternating current were more effective than those based on direct current, despite confirming that there was no movement of metals due to electroosmosis or electromigration.
- BCR sequential extraction applied to soil samples after the EKPH treatments allowed confirming that the availability of Pb and Cu increased significantly in the tailings in accordance with the findings of the soil pore water samples.
- Zn and Pb reached the highest plant concentrations but the highest bioconcentration factors were found for Cd and Cu.
- The concentrations of Pb, Cu and Cd of the ryegrass roots increased significantly with the application of AC current.
- The relatively short time of the electric current application (14 days) in relation to the total time of the experiments (58 days), along with the high initial availability of

metals in the tailings, caused the changes in the geochemical distribution of metals in the soil and their accumulation in plants were less pronounced than expected.

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FIGURE CAPTIONS

Figure 1. Experimental setup used in the electrokinetic-assisted phytoextraction test: (a) schematic representation of a container with electric current and plants, (b) images of the containers in the greenhouse during the test.

Figure 2. Daily changes in the height of the water level in both electrode wells (mean values of 14 days) for the different experimental series at three different times each day: (i) just before turning on the electric current ($t = 0$ h), (ii) just before changing the polarity of electrodes in the treatments with DC current ($t = 4$ h), and (iii) just before disconnecting the electric current ($t = 8$ h). Data are expressed as the difference between the height reached by the water in both electrode wells ($W_2 - W_1$). Different letters indicate significant differences between treatments in each sampling time ($n = 3$; Fisher's LSD test; $p \leq 0.05$). The error bars represent the standard deviation of the mean values.

Figure 3. Mean values of pH and electrical conductivity (EC) in the water from the electrode wells (W_1 and W_2) and soil pore water for the different experimental series. (a) pH (mean values of 14 days) of water in electrode wells at three different times each day: (i) just before switching on the electric current ($t = 0$ h), (ii) just before changing the polarity of electrodes in the treatments with DC current ($t = 4$ h), and (iii) just before disconnecting the electric current ($t = 8$ h); (b) EC (mean values of 14 days) of the water in electrode wells measured at the same times as the pH; (c) mean pH and EC values (of 5 samplings) of the soil pore water at $t = 8$ hours. Different letters indicate significant differences between treatments in each

sampling time ($n = 3$; Fisher's LSD test; $p \leq 0.05$). Error bars represent standard deviation of mean values.

Figure 4. Mean values ($n = 3$) of metal concentrations (mg L^{-1}) in the water from electrode wells (W_1 and W_2) and soil pore water (center area of each container) sampled just before disconnecting the electric current ($t = 8$ h) on different days of the experiment: (a) Zn; (b) Pb.

Figure 5. Geochemical fractions of Cu, Zn, Cd and Pb (% of the total concentration) in the initial growth substrate and those corresponding to the different treatments at the end of the electrokinetic-assisted phytoextraction tests. Data obtained by BCR sequential extraction: F1, exchangeable and acid-soluble metals; F2, metals bound to Fe/Mn oxides; F3, metals bound to organic matter and sulfides; and F4, metals bound to non-silicate mineral lattices). Distinct letters indicate significant differences between treatments ($n = 3$; Fisher's LSD test; $p \leq 0.05$) for each metal and fraction (F1, F2, F3 and F4).

Figure 1

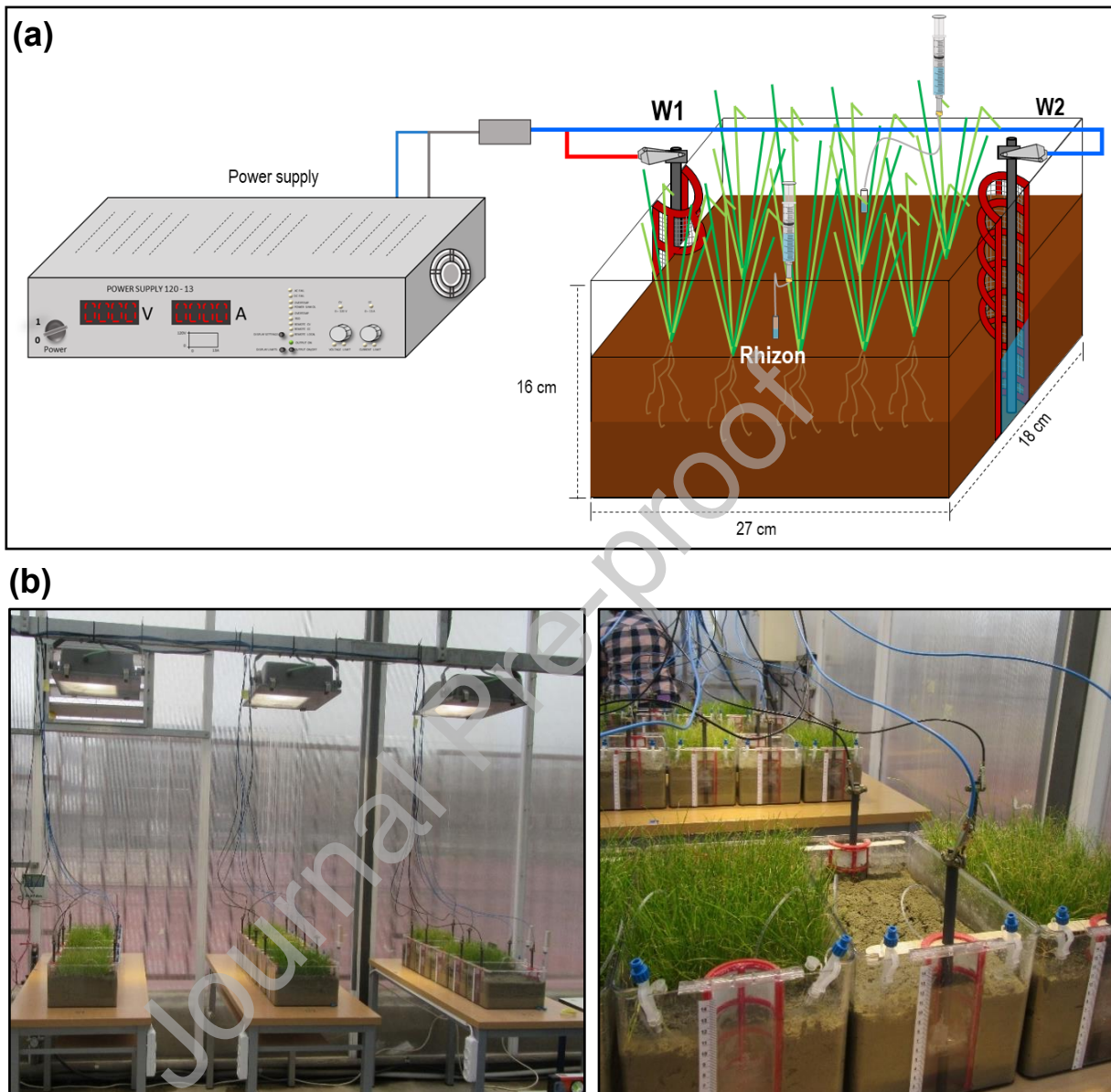


Figure 2

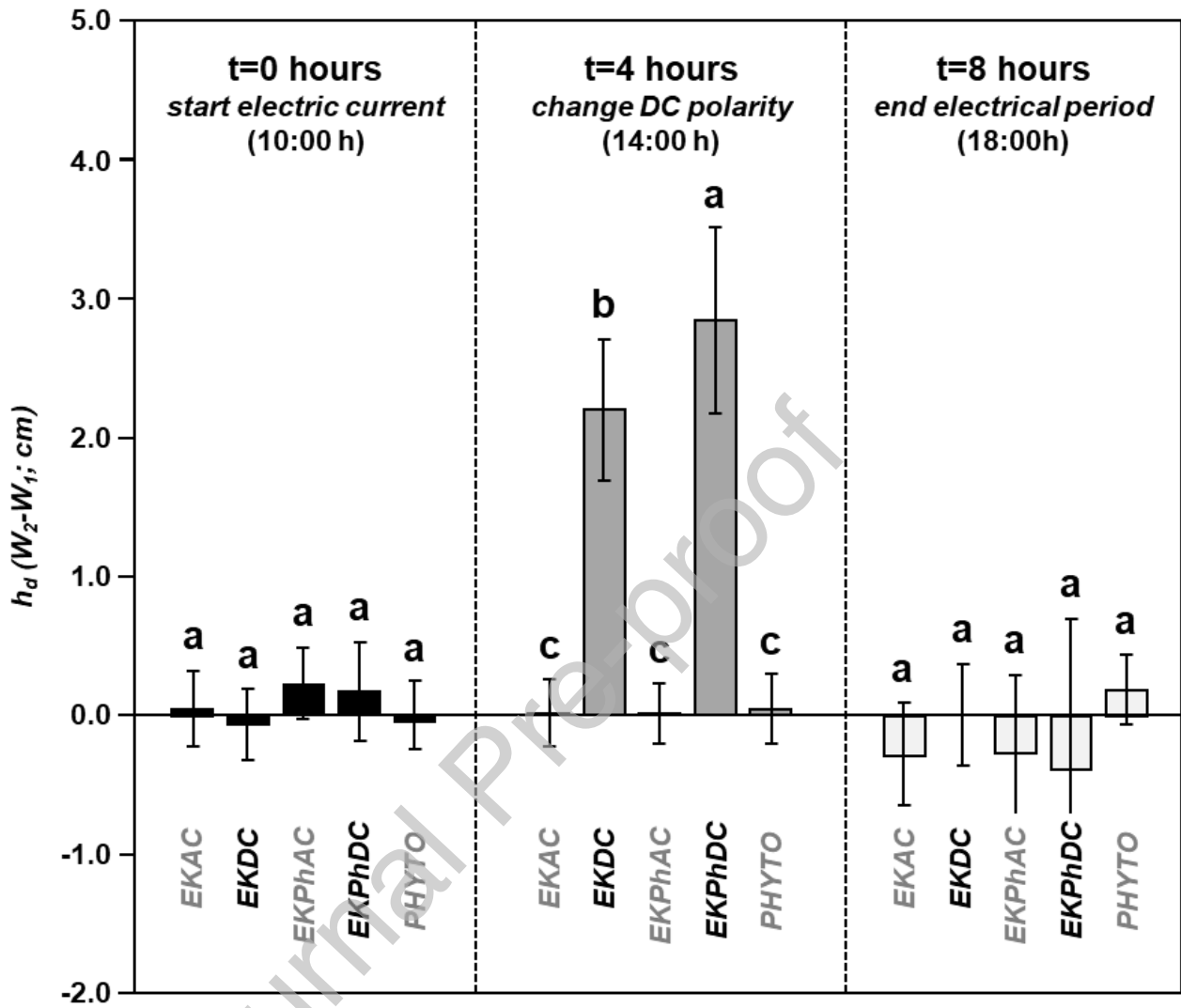


Figure 3

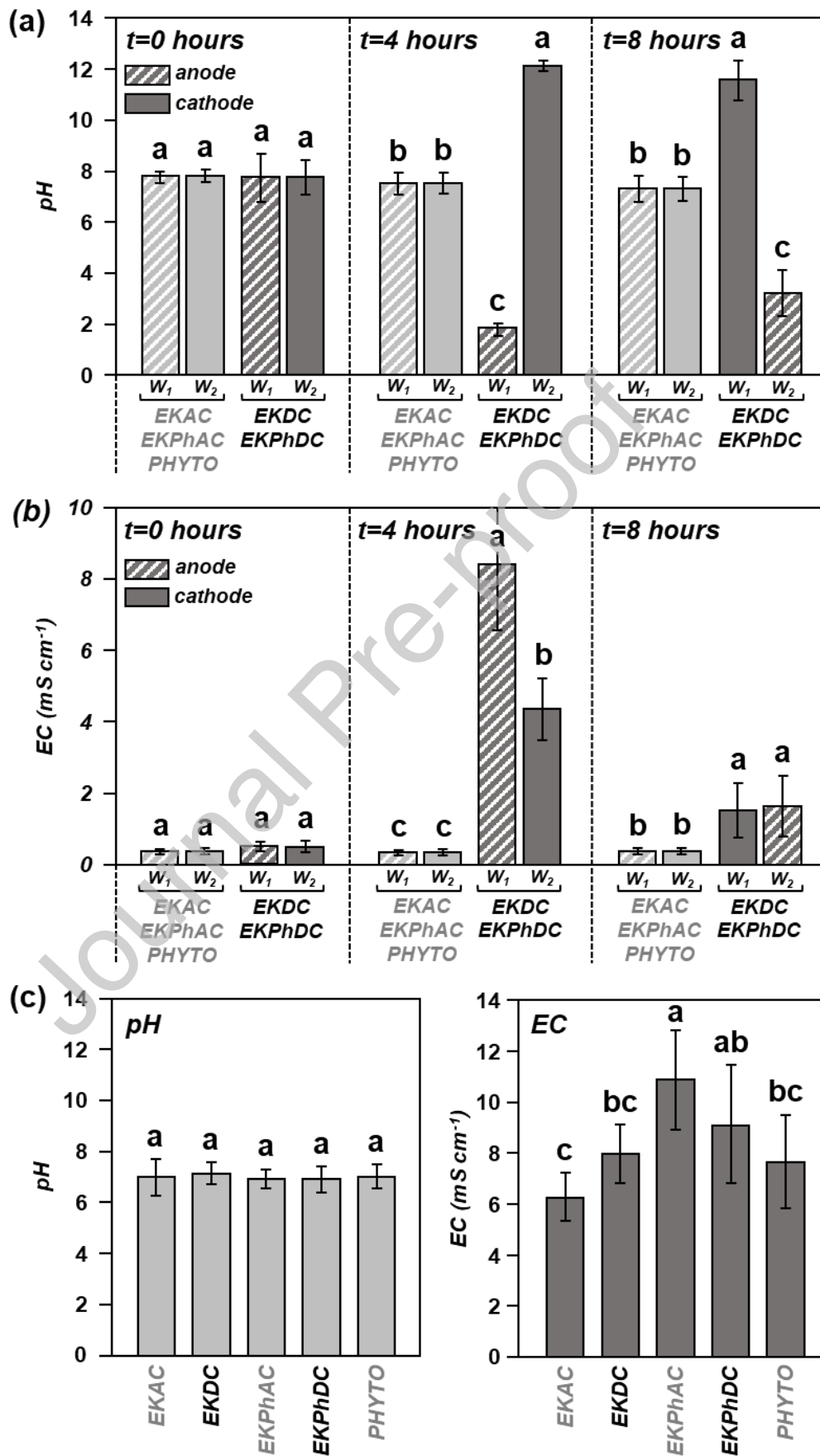


Figure 5

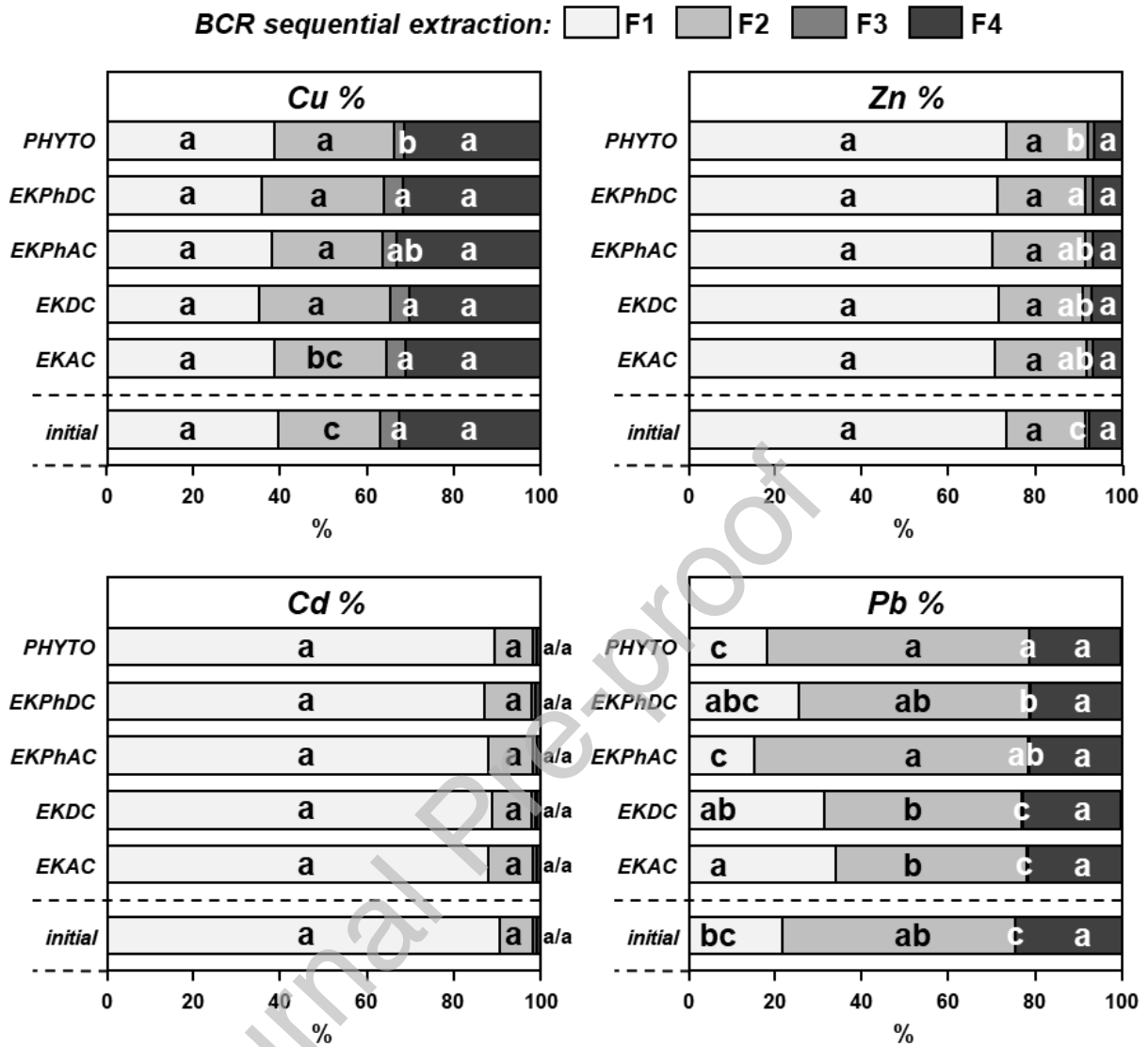


Table 1. pH and electrical conductivity (EC) of the growth substrate under different treatments (mean values \pm STD).

Treatment	pH	EC (mS cm ⁻¹)
Initial soil	6.86 \pm 0.05 a	0.68 \pm 0.31 b
EKAC	6.54 \pm 0.03 a	1.55 \pm 0.40 a
EKDC	6.66 \pm 0.29 a	1.37 \pm 0.62 a
EKPhAC	6.54 \pm 0.03 a	1.55 \pm 0.40 a
EKPhDC	6.63 \pm 0.15 a	1.99 \pm 0.80 a
PHYTO	6.78 \pm 0.20 a	1.64 \pm 0.97 a

ANOVA (LSD Test)

Sig. Level	n.s	*
Crit. Value	0.2646	1.2493

* Denote significant differences at $p < 0.05$ (Fischer's LSD test); n.s: no significant differences; STD: standard deviation. Different letters indicate significant differences between treatments (n = 3; Fisher's LSD test).

Table 2. Concentration of Pb, Zn, Cd and Cu in ryegrass tissues (roots, shoots and total plant) and phytoremediation parameters as defined in Section 3.5: translocation factor (TF) bioconcentration factor (BCF) and accumulation factor (AF) (mean values \pm STD).

Parameters	PHYTO	EKPhDC	EKPhAC
Root concentration (mg kg⁻¹)			
Pb	2339 \pm 225 c ¹	2922 \pm 180 b	3388 \pm 325 a
Zn	6924 \pm 864 a	6703 \pm 964 a	7890 \pm 730 a
Cd	784 \pm 56 b	841 \pm 103 b	1065 \pm 93 a
Cu	922 \pm 33 b	1030 \pm 97 ab	1242 \pm 145 a
Shoot concentration (mg kg⁻¹)			
Pb	58.8 \pm 14.3 a	72.3 \pm 20.4 a	64.9 \pm 17.0 a
Zn	1122 \pm 273 a	1138 \pm 184 a	1464 \pm 161 a
Cd	59.9 \pm 17.4 a	61.9 \pm 14 a	78.6 \pm 8.0 a
Cu	15.0 \pm 4.0 a	15.8 \pm 2.0 a	18.6 \pm 3.3 a
Total plant concentration (mg kg⁻¹)			
Pb	896 \pm 101 b	1078 \pm 75 ab	1262 \pm 92 a
Zn	3244 \pm 427 a	3085 \pm 62 a	3781 \pm 152 a
Cd	324 \pm 21 b	335 \pm 13 b	434 \pm 24 a
Cu	347 \pm 26 b	373 \pm 14 b	460 \pm 45 a
Translocation factor (TF)			
Pb	0.03 \pm 0.01 a	0.02 \pm 0.01 a	0.02 \pm 0.01 a
Zn	0.16 \pm 0.05 a	0.17 \pm 0.05 a	0.19 \pm 0.04 a
Cd	0.08 \pm 0.03 a	0.07 \pm 0.02 a	0.07 \pm 0.01 a
Cu	0.02 \pm 0.01 a	0.02 \pm 0.00 a	0.01 \pm 0.00 a
Bioconcentration factor (BCF)			
Pb	0.61 \pm 0.07 a	0.72 \pm 0.01 a	0.88 \pm 0.15 a
Zn	2.58 \pm 0.36 a	2.50 \pm 0.44 a	2.78 \pm 0.30 a
Cd	24.3 \pm 3.9 a	23.6 \pm 5.0 a	29.7 \pm 3.4 a
Cu	9.59 \pm 1.2 a	10.2 \pm 1.3 a	11.7 \pm 1.4 a
Accumulation factor (AF)			
Pb	0.02 \pm 0.01 a	0.02 \pm 0.00 a	0.02 \pm 0.00 a
Zn	0.41 \pm 0.08 a	0.42 \pm 0.06 a	0.52 \pm 0.08 a
Cd	1.81 \pm 0.4 a	1.70 \pm 0.30 a	2.14 \pm 0.20 a
Cu	0.16 \pm 0.02 a	0.16 \pm 0.02 a	0.18 \pm 0.04 a

¹ Different letters denote significant differences between treatments (n = 3; Fischer's LSD test; p \leq 0.05)

STD: standard deviation

Credit statements

Conceptualization and Methodology: Luis Rodríguez, Francisco J. López-Bellido, Jacinto Alonso-Azcárate and Francisco J. Fernández-Morales; Investigation: Hassay L. Medina-Díaz, Francisco J. López-Bellido and Jacinto Alonso-Azcárate; Writing, review and editing: Luis Rodríguez, Francisco J. López-Bellido and Hassay L. Medina-Díaz; Funding acquisition and Project administration: Luis Rodríguez and Francisco J. Fernández-Morales.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: