Electrokinetic-assisted phytoremediation of atrazine: differences between electrode and interelectrode soil sections

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HIGHLIGHTS

- The periodic polarity reversal of the DC field avoided marked changes in soil pH.
- Plant growth was lesser in the electrode soil sections than in the interelectrode one.
- Atrazine was transported through the soil by electromigration and electroosmotic flux.
- Electrokinetic removal of atrazine was enhanced up to 30% by maize plants.

GRAPHICAL ABSTRACT

Electrokinetic-assisted phytoremediation of atrazine: differences between electrode and interelectrode soil sections

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ABSTRACT

Electrokinetic-assisted phytoremediation (EKPR) has been recently proposed by us to remediate soils contaminated by atrazine, a common pesticide in agrarian areas. The goal of this work was to study the differences of electrochemical and biological processes occurred in the different soil sections (anode, cathode and interelectrode section) of the pots used in an EKPR test carried out with maize and a low permeability soil spiked with atrazine. An initial atrazine soil concentration of 5 mg kg^{-1} was used; the electric current (with voltage gradients of 2 and 4 V cm^{-1}) was applied during 14 days, 4 h a day and changing the polarity of the electrodes each 2 h. This operation mode avoided the extreme soil pH values, although it tended to increase throughout the EKPR test causing soil alkalisation, especially in the cathode. As a result, maize growth was poorer in the electrode soil sections. Atrazine and its metabolites, deethylatrazine and deisopropylatrazine, were taken up and accumulated in the maize tissues in a different extent depending on the soil section considered, showing the mobilization of atrazine by the electric current. The best results were found for the 2 V cm^{-1} treatment, for which the presence of maize increased 20-30% the overall atrazine removal in the cathode and middle sections of the planted pots as compared to the unplanted pots, showing the ability of EKPR to improve the efficiency of the electrokinetic remediation. Electromigration and electroosmotic flux contributed to transport atrazine and its metabolites throughout the soil.

Keywords: atrazine, maize, electrokinetic-assisted phytoremediation, electroosmosis, electromigration

1. INTRODUCTION

The excessive use of pesticides, as atrazine herbicide, may cause severe environmental contamination problems since these chemicals may pose health risks to humans owing to its mutagenic and carcinogenic activity [1,2]. Therefore, it is necessary to develop efficient and sustainable technologies for the remediation of soils and waters contaminated by this type of compounds. The coupling of electrokinetic remediation and phytoremediation, called as electrokinetic-assisted phytoremediation (EKPR), has been studied in the field of the soils contaminated by heavy metals [3,4,5,6,7,8,9]. However, little is currently known about its application to soils polluted by organic pollutants. To our knowledge, there are only two papers which have reported, without conclusive results, the application of EKPR to soils polluted by heavy metals and PAHs [10,11]. More recently, Sánchez et al. [12] studied the application of EKPR to a soil spiked with atrazine using maize plants. They found that the application of an electric field significantly enhanced the atrazine removal with respect to that of a phytoremediation test, although the application of the electric current led to a slight decrease on maize biomass.

According to O'Connor et al. [3], the key factor in EKPR is the electrokinetic mobilisation of some chemical species in the soil, which increases its availability to plant roots leading to a greater uptake/degradation by plants (rhizodegradation and/or phytodegradation). This technology is particularly interesting for low permeability soils where the movement of water and dissolved pollutants is partially hindered. Different physical and chemical phenomena occur during EK-phytoremediation. Electromigration, electroosmosis and electrophoresis are the physical processes which take place, while electrolysis and precipitation would be the main involved chemical ones [13,14,15,16].

Electromigration is the transport of dissolved ionic species toward the opposite-charged electrode. Electroosmosis is the movement of the soil pore water, with dissolved ionic and non-ionic species, relative to a stationary charged surface (soil particles) by the action of an electric field. Electrophoresis is the opposite to electroosmosis, that is, the transport of charged particles of colloidal size relative to a stationary pore fluid. In addition, other processes as desorption, dissolution and chemical interactions with the soil must also be taken into account [17]. Finally, electrolysis of water at the electrodes produces H^+ ions at the anode (oxidation) and OH^- at the cathode (reduction) [15]; these phenomena have a critical influence on the changes in soil pH which are usually found in the electrokinetic remediation of soils [18]. The pH variation of the soil system due to the water electrolysis reactions and the particular conditions generated at the electrodes are of particular interest in the case of EKPR technology, since they could represent unfavourable conditions for plant growth, which need to be studied.

This work is part of a wider research project focused on the use of EKPR to decontaminate soils polluted with pesticides. Previously, atrazine was selected as a model of moderately polar herbicide (octanol partition coefficient below 3 and water solubility of 33 mg.L^{-1}) [19], indicating the potential of atrazine for its mobilization in the environment [20]. Although its use is currently banned in Europa [21], it is still largely used in the USA, China, and Australia [22]. On the other hand, maize plant (*Zea mays* L.) was selected as an adequate plant species for EKPR on the basis of its tolerance and resistance to atrazine [23]. The goal of this work was to study the differences of electrochemical and biological processes occurred in the different soil sections of the pots used in an EKPR test carried out with maize and a soil spiked with atrazine, with particular emphasis on the changes

in soil pH, plant biomass, atrazine uptake and accumulation by plants and the removal and/or degradation of atrazine in soil.

2. MATERIALS AND METHODS

2.1. Materials

TABLE 1

The soil used in this study was an unpolluted natural soil coming from a quarry used for brick production located in Mora de Toledo (Toledo, Central Spain; UTM coordinates X: 431366 Y: 4395232). It was collected from a depth between 0.5 and 1 m. The soil was air-dried in the laboratory during 3 days at a temperature around 20°C, and then sieved through a nylon sieve (Ø 2 mm) in order to remove residues prior to the experiment. The main physicochemical properties were analysed by means of normalized methods; they are shown in Table 1. The soil had a pH of 9.42 and it was classified as a low plasticity clay (CL) soil according to the Unified Soil Classifications System (USCS). Additionally, the acid/base buffer capacity of the soil was determined according to the method described by Yeung et al. [24]. Dry soil samples of 1.5 g each were placed in centrifuge tubes and 30 mL of deionized water was added to each sample. The soil mixtures were thoroughly shaken for 24 h, then small accurately measured volumes of 1 M HNO_{3(aq)} or 1 M NaOH_(aq) (25 µL) were added to each soil mixture. The samples were shaken again for another 24 h and the pH of the soil mixtures were measured. Under the given experimental conditions, the acid/base buffer capacity of the soil used was very high, since the addition of acid or base only changed the soil pH in the range 6-11 (Figure S1).

A commercial variety of *Zea mays* L. (dent corn hybrid cultivar) was used in the experiment. Seeds were pre-germinated in a growing medium (0.5 mM CaSO₄) and kept moist during three days at 28°C. After the germination period, healthy seeds with uniform size were selected and carefully transplanted to the soil. Solid atrazine of analytical standard quality (Fluka, analytical standard 99.1%) was used to prepare the aqueous solutions which were added to the pots.

2.2. Experimental design

FIGURE 1

The soil (1.0 kg of dry soil per pot) was packed into cylindrical plastic pots (16 cm diameter and 15 cm height). The soil of each pot was divided in three sections by using vertical nylon meshes (35 µm pore size) and labelled as left (0-5 cm), middle (5-11 cm) and right (11-16 cm) sections along with the pot diameter (Figure 1). There was 300 g of soil in the electrode (left and the right) sections and 400 g of soil in the interelectrode (middle) section. Pregerminated maize seedlings were transplanted into the pots at a rate of 4 individual plants for left and right soil sections and 6 individuals for the middle section, in order to ensure a similar growing space for all the plants. The pots were grown in a growth chamber using temperatures of 27 °C/ 16° C day/night and an approximately constant soil moisture of 60% of water holding capacity (controlled by weighing the pots).

In the day 35 after planting, aqueous solutions of atrazine were homogeneously sprayed on the soil surface of the pots at a rate of 5 mg of atrazine per kg of soil (corresponding to 23.18 µmol per pot). Electrical DC current (2 or 4 V cm⁻¹) was switched on

1 immediately after atrazine spiking using a pair of electrodes and a DC power supply
2 (Delta Elektronika S.V., model SM120-13, 0-120 V: 0-13 A). Graphite rods (length 8 cm
3 and diameter 6 mm) were used because their inertness; they were vertically inserted into
4 the soil at both sides of the pots and connected to the DC power supply by means of metal
5 clamps attached to the free upper part of the electrodes. The operation mode used for
6 electric current application was 4 h a day and included the reversal of polarity every 2 h.
7 The total duration of the EKPR test was 14 days from the application of atrazine and the
8 electric field. The experimental conditions were based on our previous research [12,23].

9
10 Four different treatments were used in this experiment: (i) pots with maize plants and
11 application of a 2 V cm⁻¹ electric field; (ii) pots with maize plants and application of a 4
12 V cm⁻¹ electric field; (iii) pots without plants (control) and application of a 2 V cm⁻¹
13 electric field; (iv) pots without plants (control) and application of a 4 V cm⁻¹ electric field.
14 All the treatments were carried out in triplicate.

15
16 Soil surface samples were taken and analysed for pH at 4, 28, 148 and 316 hours after the
17 beginning of the experiment, immediately after the disconnection of the electric current.
18 Separate soil and plant samples were collected at the end of the experiment from the three
19 sections (left, middle and right) in which the pots were divided in order to determine the
20 atrazine (and its metabolites) concentration remaining in the soil and its uptake and
21 accumulation in maize plants (roots and shoots). Maize shoots were harvested by cutting
22 the stem 1 cm above the soil surface. Roots were harvested by carefully separating the
23 biomass from the soil and rinsed them thoroughly with deionized water. Plant samples
24 were air-dried for 5 days and homogenized using a grinder (Retsch Model MM2000,

Germany). The dry plant biomass was recorded. Soil and plant moisture values were determined with the gravimetric method using an oven for 24 h at 105 °C.

2.3. Atrazine extraction and analysis

The extraction of soil and plant samples was carried out by using a method based on the reported by Amadori et al. [25]. 3 mL of acetonitrile (Acetonitrile Chromasolv for HPLC, gradient grade, Sigma-Aldrich, USA) were added to 2.0 g of sample in 25 mL glass flasks. The flasks were covered and shaken for 30 min on a rotary shaker. Then, the suspensions were centrifuged and the supernatant phases were recovered and reserved. The extraction step was repeated three times and the respective supernatant phases were blended. The extracts were filtered through a 0.45 µm nylon membrane before analysis. Atrazine (ATR) and its main metabolites (DEA: deethylatrazine, and DIA: deisopropylatrazine) were determined by high performance liquid chromatography (HPLC). A Shimadzu Prominence UFLC XR (Japan) chromatograph equipped with a C18 reverse-phased column (240 mm x 4 mm) was used for determining atrazine and its metabolites in the extracts. The operating conditions were the following: acetonitrile and 0.01 M phosphate buffer solution (pH = 7.1) as mobile phase, a flow rate of 0.5 mL.min⁻¹ for 25 min, an oven temperature of 30 °C and a sample injection volume of 20 µL. The retention times of atrazine, deethylatrazine and deisopropylatrazine were 16.1, 7.9 and 4.5 min, respectively.

TABLE 2

Concentrations of ATR, DEA and DIA in soils and plants have been expressed in the paper as μmol (per kg of soil or plant) instead μg , due to the different molecular weight of the atrazine and its metabolites (Table 2).

2.4. Statistical analysis

The SPSS 22 Statistical Software was used for the statistical analysis. ANOVA analysis was used to compare if there were statistically significant differences among the mean values of the data from the different soil sections (Duncan test, $p < 0.05$). Normality of the data was previously checked by using the Kolmogorov-Smirnov test.

3. RESULTS AND DISCUSSION

3.1. Soil pH distribution

FIGURE 2

In Figure 2 it can be seen the evolution of the soil pH corresponding to the three sections of the pots (left, middle and right), measured immediately after the disconnection of the electric current in the days: 1st, 2nd, 7th and 14th from the beginning of the EKPR test. Those measures correspond to 4, 28, 148 and 316 h (from the beginning of the EKPR test) in the Figure 2.

Soil pH varied in the range 8.49-10.46 for all the treatments, that is, less than a unit as compared to the initial soil pH (9.42). Therefore, the periodical changes in the polarity of

the electric field, together to the low application time (4 h a day), had the desired effect of avoiding the characteristic extreme acidic and alkaline conditions generated in the electrode regions of the electrochemical processes without polarity reversal [8,9,15,16].

It is very important to point out that the control of the soil pH is a necessary condition for the practical application of the EKPR technology. Other strategies to avoid extreme soil pHs, different than periodical polarity reversal [27,28], can be found in the previous literature, e.g. cathode chamber washing [29], the use of ion-exchange membranes to separate electrode chambers [30] and the use of special electrode configurations [16].

It can be also observed that, for all the treatments, soil pH values in the left section were higher than those of the right section (Figure 2). This is a consequence of the electrochemical role of the both soil sections at the precise moment when the pH was measured (just after the disconnection of the electric current). Every day, when DC was switched on, left and right electrodes began to work as anode and cathode, respectively; however, due to the polarity change after 2 h, when DC was switched off, the role of the left and right electrodes was cathode and anode, respectively. Therefore, the higher pH values in the left section of the pots may be explained by taking into account the electrolysis reactions occurring in the anode and cathode, which generate hydrogen (H^+) and hydroxyl (OH^-) ions, respectively [14].

Another observed trend was the increase in soil pH as the experiment progressed (Figure 2). The high buffer capacity of the soil used in this study avoided the acidification of the soil, especially in the anode (Figure S1), which was particularly effective in the neutralization of protons generated in the anode as seen by the alkaline pH values of both electrode sections at the end of the experiment (316 h). Nevertheless, since the electric

current was only applied 4 h a day, it is expected that other phenomena, i.e. the diffusive transport of ions and water in the unplanted pots and/or the advective transport –induced by maize roots in planted pots- also contribute to counteract the pH changes caused by the DC application.

The presence of maize plants led, in general, to lower soil pH values as compared to non-planted pots. More specifically, soil pH in planted pots varied in the range 8.49-10.03 while the range for non-planted pots was 8.76-10.46 (Figure 2). It may be explained by the release of root exudates, consisting of sugars, amino acids and organic acids such as citric, oxalic, malic, fumaric and acetic by the maize roots [31,32]; it is considered as a common mechanism used by plants to modify the rhizosphere in order to acquire nutrients [33]. Lastly, the presence of maize plants must be the responsible of the alteration in the pattern observed for the pH variation in the soils corresponding to non-planted pots: left>middle>right. In fact, for planted pots, the soil pH values in the middle section were lower, e.g. for the 2 V cm⁻¹ treatment, or even higher, e.g. the pH values corresponding to 4 and 28 h in the 4 V cm⁻¹ treatment, than those of the electrode sections. This contradictory behaviour can only be explained as a result of the heterogeneity in the local soil conditions. Thus, factors such as the presence of maize roots and the known non-uniform pore fluid environment caused by the electric field throughout the experimental soil specimens [18] could explain the abovementioned heterogeneity. Moreover, it has to be considered that pH changes in soil pore water could not be immediately detectable in the solid soil phase.

3.2. Effect of electrochemical processes on plant growth

FIGURE 3

The biomass production of the maize plants in all the soil sections is shown in Figure 3. The data are expressed as g plant⁻¹ because of the variable number of plants sown in the different soil sections, i.e. 4 and 6 plants for the electrode and the middle sections, respectively (see Section 2.2).

Total biomass yielded between 0.41 and 0.56 g plant⁻¹ for all the treatments and soil sections. According to the intrinsic characteristics of the maize, root biomass values were lower than those of the shoots for all the soil sections and the treatments. Root biomass ranged between 0.14 and 0.20 g plant⁻¹ while shoot biomass did between 0.26 and 0.36 g plant⁻¹ (Figure 3). In a previous paper, we reported that the application of an electric current of 2 and 4 V cm⁻¹ only decreased significantly, as compared with pots without DC application, the root biomass of maize (the plants were grown in the interelectrode soil section), while shoot biomass was not significantly affected by the electric field [12]. However, the results presented here show that shoot and total biomass values corresponding to the electrode sections were significantly lower than those of the middle soil section for all the treatments (with the exception of the right section in the 2 V cm⁻¹ treatment). Root biomass corresponding to the middle soil section was also significantly higher than those of the electrode sections for the 2 V cm⁻¹ treatment, although it remained similar in all the soil sections for the 4 V cm⁻¹ treatment.

1 Therefore, our results show that the processes occurring in the soil zone close to the
2 electrodes negatively affected plant growth, leading to significant changes in the plant
3 behaviour with respect to the soil located between the electrode sections. The application
4 of an electric field should not affect plant growth by itself; however, its indirect effects
5 on soil properties (pollutant concentration, soil pH and its influence on nutrient
6 bioavailability, etc.) may be decisive [3]. Thus, two effects could be suggested to explain
7 the decrease in plant biomass: on one hand, the soil pH changes caused by electrode
8 electrochemical processes and, on the other hand, the differences in atrazine
9 concentration of the plants belonging to the different soil sections.

10
11 Regarding pH changes, we found significant negative correlations between soil pH and
12 root and shoot biomass (Pearson's coefficients of -0.601 and -0.760 for root and shoot
13 biomass, respectively, $p < 0.01$). Therefore, although the observed changes in soil pH were
14 relatively moderate (Section 3.1), it seems that soil alkalinisation damaged maize growth
15 in some extent. It has been reported that high values of soil pH can lead to a blockage of
16 nutrients as important as iron for maize [34]. Moreover, this hypothesis would also
17 explain the differences in plant biomass between left and right electrode sections. As it
18 can be seen in Figure 3, for the 2 V cm^{-1} treatment, total plant biomass from left soil
19 section was approximately 18.5% lower than that of the right soil section. However, total
20 plant biomass of left and right soil sections corresponding to the highest voltage gradient
21 (4 V cm^{-1}) were not statistically different. The results from the low voltage treatment may
22 be surprising because the periodic changes of polarity should lead to a similar behaviour
23 in both electrode soil sections. However, we have already shown the higher tendency to
24 alkalinisation of the left electrode with respect to the right one (Section 3.1); it would
25 explain the differences in plant biomass observed for the 2 V cm^{-1} . In the case of the 4 V

cm⁻¹ treatment, the higher extent of the pH changes would lead to a detrimental effect on plant growth with a similar magnitude in both electrode sections.

The potential effect of the atrazine plant accumulation on the maize growth will be discussed in the next Section (3.3).

3.3. Accumulation of atrazine in plant tissues

FIGURE 4

Figure 4 shows the concentration of atrazine residues (this term refers to both atrazine and its metabolites, deethylatrazine and deisopropylatrazine), in roots, shoots and total plant for all the soil sections at the end of the experiment. Plant uptake and accumulation of atrazine and its metabolites was observed for all the plant tissues and soil sections, showing the contribution of maize plants to soil decontamination in some extent. Most of the atrazine accumulated in maize tissues corresponded to the metabolites deethylatrazine (DEA) and deisopropylatrazine (DIA), which are the main metabolites detected in plant after atrazine uptake [35,36]. Although atrazine metabolites may have been produced inside the plant tissues (phytodegradation) and/or come from the microbial degradation occurred in rhizosphere (rhizodegradation) [22,36,37,38], the much higher proportion of metabolites in the plants than in the soil (see next section) suggested that significant biodegradation occurred within the maize plants. It agrees with our previous results [23] and those of other researchers [36]. On the other hand, atrazine residues accumulation occurred mainly in roots for all the treatments and soil sections (Figure 4,

onset); the same trend was previously found for atrazine phytoremediation without using electric current [39].

Overall, the accumulation of ATR residues in plants was different depending on the soil section and the voltage applied. For the lowest voltage, the order for the ATR residues concentration in maize was middle>right>left, while for the 4 V cm⁻¹ treatment, the order was right>middle>left (Figure 4). Therefore, since those trends do not agree with the biomass variations described in the previous section (Section 3.2), it can be said that the change in soil pH at the vicinity of the electrodes was the most important parameter influencing plant growth in this experiment. On the other hand, if we consider plant accumulation as an indicator of atrazine (and its metabolites) mobility in soils, we can conclude that the application of an electric field led to the effective transport of ATR residues in the soil. As a result, atrazine uptake was significantly higher in the middle soil sections, as compared to those of the electrode sections, in the pots belonging to the 2 V cm⁻¹ treatment. However, for the 4 V cm⁻¹ treatment, the highest plant concentration was found for the right electrode; it could indicate that atrazine remains more time in the right soil section than in the others. It will be next discussed in the Section 3.4.

3.4. ATR residues remaining in the different soil sections

FIGURE 5

In Figure 5 are shown the atrazine, DEA and DIA amounts (expressed as a fraction of the initial atrazine in the respective soil sections) remaining in soils after the EKPR

experiment, together with the overall percentage of atrazine removal and the atrazine residues (ATR+DEA+DIA) accumulated in plant tissues.

The effect of maize plants on atrazine removal from soil was different for the two voltages applied and for the different soil sections. While for the 2 V cm⁻¹ treatment the presence of maize significantly improved ATR removal in the middle and left soil sections (but not for the right section), no significant enhancement was found for the highest voltage in all the soil sections. It means that other processes, in addition to plant uptake, contribute to the overall atrazine removal from soils. In fact, the plant accumulation of ATR residues ranged between 1.07 and 3.75% of the initial parent atrazine (Figure 5). Those mechanisms could be rhizosphere microbial degradation [23] and volatilization [40,41].

Microbial degradation of atrazine in soils has been previously reported [36,37,38,42], being the N-dealkylation the preferred degradation pathway in soils [42]. This is the reason for which we have focused on the metabolites DEA and DIA, although the presence of other metabolites, coming from other biologically-mediated pathways, such as hydroxyatrazine (HA), cannot be completely discarded; anyway, no significant chromatographic peaks identified as HA were found in our experiment. Therefore, the presence of DEA and DIA in all the soil sections at the end of the EKPR experiment is indicative that microbial degradation occurred in some extent. Moreover, the values of the ratio (DEA+DIA)/ATR in planted pots were up to 4-fold times higher than those from unplanted (control) ones (Figure 5), which strongly indicates the role of maize roots to stimulate microbial growth and atrazine degradation. Since the values of this ratio were higher for the control pots belonging to the 4 V cm⁻¹ treatment than those of the 2 V cm⁻¹, it means that microbial activities could be enhanced when the applied voltage was

increased; Gill et al. [43] reported that low electric current has positive effects associated with the generation of oxidising and reducing zones favourable for biodegradation of contaminants close to the electrodes.

Partial volatilization of atrazine can also take place in some extent due to the moderate vapour pressure of atrazine and the locally high temperatures in the soil directly in contact with electrodes [40,41]; however, the short period of time in which electric field was applied and other results recently reported by us [12] seems to indicate that this mechanism of atrazine removal was not relevant in the present EKPR experiment. According to the results in the onset of Figure 5, it seems that phytodegradation of atrazine is only relevant when reduced voltage gradients (2 V cm^{-1}) are used in EKPR systems; however, for higher voltages, other mechanisms, such as volatilization and microbial degradation, could be the most important processes for atrazine removal from soils.

Atrazine, DEA and DIA have pKa values in the range 1.30-1.71 (Table 2); so, their amino groups may be partially ionized at alkaline pHs leading to the corresponding negatively charged species [44]. Those anionic species could be transported to the anode by means of electromigration. On the other hand, ATR residues can be partially dissolved in water (Table 2); so, they could be also transported under the electric field by the electroosmotic flux. Soil particles are usually negatively charged due to isomorphic substitution and broken bonds; so, there will be a net excess of cations in the fluid layer in contact with the soil. The movement from the anode to the cathode of those cations causes the momentum transfer to the soil water. As a consequence, electroosmotic flux usually flows from the anode to the cathode, although it may be reverted under specific soil conditions

[18]. In our case, taking into account that, on one hand, the clay fraction of the soil used here is mainly composed of smectite and illite (Table 1), therefore being considered as a permanent-charge soil, and, on the other hand, the soil pH remained in high values, the electroosmotic flux is expected to be from the anode to the cathode throughout the entire EKPR test [18]. Therefore, electromigration and electroosmosis would transport atrazine and its metabolites in opposite directions under the electric field application. As a result of this effective transport from one electrode to the other, the highest efficiency on atrazine removal should be found for the middle sections of the pots, which strongly agrees with the results shown in Figure 5. It is remarkable the supplementary increase in atrazine removal caused by maize plants in the middle section of the pots corresponding to the 2 V cm^{-1} treatment, in which the pollutant uptake by the maize plants could have contributed significantly to the overall atrazine removal.

In the electrode sections, the concentration of atrazine residues in the plant tissues should be closely related with the accumulation of those compounds in the soil. Thus, for the 2 V cm^{-1} treatment, the highest ATR residues plant concentrations were found for the left electrode (considering only electrode sections, Figure 4), which acted as a cathode when the electric current was disconnected every day. Therefore, the electroosmotic transport of ATR residues predominated at this voltage, leading to a higher residence time of pollutants in the left (cathode) section with subsequent higher ATR residues plant concentrations and lower soil concentrations (Figure 5). On the contrary, for the 4 V cm^{-1} treatment, electromigration predominated over the electroosmotic transport, leading to a higher plant accumulation and lower soil concentrations for the right electrode (which acted as anode when the electric current was switched off daily). This latter fact agrees with the reported fact that electromigration grows more than electroosmotic flux when

current intensity is increased [45]. It is important to highlight that both electroosmotic and electromigration fluxes could not be measured in this EKPR test because no electrolyte wells were used and the soil was not water-saturated in order to avoid damages to plant growth.

4. CONCLUSIONS

The main conclusions obtained from this study are the following:

- It has been shown that the periodical changes in the polarity of the electric field is a satisfactory strategy to avoid extreme soil pH values, which could damage plant growth and compromise the ability of EKPR. Nevertheless, soil pH tended to increase over time causing soil alkalisation, which was more important in the left electrode (cathode).
- Plant growth was lesser in the electrode soil sections than in the soil located between them; it was due to changes in soil properties, e.g. soil pH, pollutant concentrations and nutrients bioavailability, which occurred near the electrodes. More specifically, maize growth was more affected for the 4 V cm⁻¹ treatment mainly due to the increasing soil pH in the left (cathode) area.
- Atrazine and its metabolites, deethylatrazine and deisopropylatrazine, were mainly produced within the plant and accumulated in the maize tissues; the different concentrations found for the maize plants grown in the different soil sections showed the mobilization of atrazine by the electric current. For the lowest voltage, the order for the ATR residues concentration in maize was

1 middle>right>left, while for the 4 V cm⁻¹ treatment the order was
2 right>middle>left.

- 3 - The presence of maize increased 20-30% the overall atrazine removal in the left
4 and middle sections of the planted pots as compared to the unplanted pots in the
5 2 V cm⁻¹ treatment, showing the ability of EKPR to improve the efficiency of the
6 electrokinetic remediation.
- 7 - Atrazine removal efficiency was different depending on both the soil section and
8 the voltage applied, which could be related to the electrochemical processes taking
9 place in the soil.

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Table 1. Main physicochemical properties of the soil used in the EKPR experiment.

Soil property	Value	Method
Saturation degree (%)	33	ASTM Standards D-2980
pH	9.42	1:5 soil/water mixture
Soil organic carbon content (%)	0.60	Total organic carbon analyser
CEC (cmol.Kg ⁻¹)	23.41	Ammonium acetate saturation method
Bulk density (g.cm ⁻³)	1.54	ASTM Standards D-854
Liquid limit (%)	42	
Plastic limit (%)	24	ASTM Standards D-4318
Plasticity index (%)	18	
Clay mineralogy		
Smectite (%)	50-75	X-ray diffraction of oriented aggregates
Illite (%)	25-50	
Kaolinite (%)	0-25	
USCS Classification CL (a low plasticity clay)		

Table 2. Physical-chemical characteristics of atrazine and its main metabolites
[19,26].

Compound	Property			Chemical structure
	Molecular mass (g mol ⁻¹)	Water solubility (mg L ⁻¹)	pK _a	
Atrazine (ATR)	215.68	33	1.68-1.71
Deethylatrazine (DEA)	187.63	3200	1.30-1.65
Deisopropylatrazine (DIA)	173.60	670	1.30-1.58

FIGURE CAPTIONS

Figure 1. Experimental setup of the electrokinetic-assisted phytoremediation test.

Figure 2. Soil pH measured at 4, 28, 148 and 316 hours from the beginning of the EKPR test in the three soil sections. Error bars represent the SD of three replicates.

Figure 3. Root, shoot and total plant biomass (g-dry-biomass plant⁻¹) by soil section.

Error bars represent the SD of three replicates. The different letters mean significant differences between total plant biomass in the different soil sections ($p < 0.05$; Duncan test).

Figure 4. Total atrazine uptake ($\mu\text{mol kg}^{-1}$ dry biomass) and the distribution of the atrazine residues (ATR: atrazine; DEA: deethylatrazine; DIA: deisopropylatrazine) in roots, shoots and total plant at the end of the EKPR experiment by soil sections.

Error bars represent the SD of three replicates. Different letters indicate significant differences between soil sections ($p < 0.05$; Duncan test).

Figure 5. Distribution of atrazine, DEA and DIA, expressed as a fraction of the initial atrazine, remaining in the different soil sections at the end of the EKPR experiment.

Inset: relationship between ATR metabolites and ATR concentrations remaining in soils, overall atrazine removal in soils (% of the initial ATR) and ATR residues plant accumulation (% of the initial ATR).

1 **Figure S1. Buffer capacity of the soil used in the EKPR experiment. pH reached by**
2 **the soil vs added volume of acid (Δ) and base ($\bullet$)**

3

4 **Figure S2. Chromatograph of the calibration solution used for the HPLC analysis.**

5

FIGURE 1

FIGURE 2

FIGURE 3

■ Root ■ Shoot — Total plant (root+shoot)

FIGURE 4

FIGURE 5

Voltage gradient (V cm ⁻¹)	Soil section	Pot	(DEA+DIA)/ATR in soil (mol mol ⁻¹)	Atrazine removal (%)	(ATR+DEA+DIA) in plants (%)
2	Left	Maize	0.37±0.04	66.50±2.91	1.50±0.05
		Control	0.07±0.00	34.96±1.63	-
	Middle	Maize	0.23±0.05	67.18±6.26	3.75±0.04
		Control	0.11±0.01	45.46±1.84	-
	Right	Maize	0.08±0.01	25.53±0.97	1.51±0.02
		Control	0.08±0.00	39.36±3.19	-
5	Left	Maize	0.26±0.01	40.06±0.52	1.07±0.02
		Control	0.10±0.00	42.54±2.99	-
	Middle	Maize	0.36±0.05	73.80±3.72	1.82±0.03
		Control	0.24±0.01	74.28±0.68	-
	Right	Maize	0.36±0.02	60.16±0.92	1.52±0.01
		Control	0.15±0.01	57.62±0.13	-

FIGURE S1

FIGURE S2