**A mesocosm study of electrokinetic-assisted phytoremediation of
atrazine-polluted soils**

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HIGHLIGHTS

EKPR of atrazine-polluted soils is feasible at mesocosm scale

Atrazine was transported in the soil by electro-osmosis and electromigration

Final distribution of atrazine in soils agrees with its concentration in soil pore water

Some limitations relative to the scaling up of EKPR processes have been detected

ABSTRACT

Phytoremediation, an ecological remediation technology based on the use of plants, can be combined with electrokinetic treatment for the removal of both inorganic and organic pollutants from soils. Electrokinetic-assisted phytoremediation (EKPR) of atrazine was tested in a mesocosm scale experiment using ryegrass (*Lolium perenne* L.). Two mock-ups with dimensions of 2.25 (L) x 0.49 (W) x 0.50 (H) m were initially filled with an unpolluted low plasticity clay soil and used to carry out an EKPR test and another electrokinetic remediation (EKR) one after spiking the soil with atrazine at a dose of 2 mg kg⁻¹. Experiments were conducted for 19 days using a DC electrical field of 0.6 V cm⁻¹ applied continuously without changing polarity. Samples of soil, plants and soil pore water corresponding to ten different soil sections of each mock-up were taken and analysed throughout the experiment. Soil pH followed an increasing profile from the anode to the cathode, although it kept moderate values in the range of 7.53-9.62. Electro-osmosis, gravity and plant roots (in the EKPR series) influenced the final distribution of water and atrazine in the soil. Additionally, the electromigration flux had a relevant role in the atrazine residues transport during the EKPR process. Concentration values of atrazine residues remaining in the different soil sections were in good agreement with those of the soil pore water, with most of the atrazine being accumulated in the cathode section. Atrazine was mainly removed from soils by biochemical degradation, which was greatly improved by ryegrass plants; the overall atrazine removal yield was increased from 40.20% (unplanted mock-up) to 61.01% (planted mock-up).

Keywords: atrazine; ryegrass; electrokinetic-assisted phytoremediation; scaling-up; soil

1. INTRODUCTION

Soil pollution is a hidden threat for human health, food security and the environment. Due to the their intensive application on agricultural soils, a large amount of pesticides, such as atrazine, are accumulated and further released from soils to the atmosphere, groundwaters and living organisms [1]. Therefore, environmental scientists need to research and develop soil remediation technologies that are environmentally sustainable. The combination of electrokinetic remediation and phytoremediation (electrokinetic-assisted phytoremediation, EKPR) has been recently set forth to overcome the current drawbacks of the individual technologies. Thus, while plants carry out the degradation and/or accumulation of the pollutants, the application of a low intensity electric field to the soil favours their mobility and bioavailability making the remediation process more efficient [2]. Electrokinetic treatment of the soil involves several processes such as the electromigration of the charged ionic species towards the electrodes of opposite charge and the movement of pore water, with the subsequent drag of non-charged pollutants, by electro-osmosis [3–5]. Together with those transport processes, electrolysis reactions occur at electrodes (Eqs. (1) and (2)), resulting in a pH jump in the pore fluid [6].

All the processes described above contribute to the movement of pollutants throughout the soil, being possible to solve some of the main disadvantages of phytoremediation, such as the limitations from root depth and density which influence the contact between root and pollutants, the long period of time that remediation needs, etc. [7,8]. The evident

ecological benefits of phytoremediation together with the consideration of the electrokinetic remediation as a cost-efficient technology (the electric power could be supplied by photovoltaic cells) make EKPR a very promising on-site technology for polluted soils.

To date, EKPR has been mainly applied to soils polluted by heavy metals [9–14], while few attention has been paid to organic pollutants. Chirakkara et al. [15] and Acosta-Santoyo et al. [16] applied the combined EKPR technology to a mixed contaminated soil (by heavy metals and polycyclic aromatic hydrocarbons), although they did not find a significant improvement in the removal efficiency as compared to that of phytoremediation experiments. However, we have recently shown that a moderate electric field (1-2 V cm⁻¹) can significantly improve the atrazine removal by maize and ryegrass [17–19]; Rocha et al. [20] have reported an enhancement of removal efficiency and plant biomass by using EKPR with reversal polarity and maize for the treatment of a soil spiked with petroleum. The relevance of the abovementioned results highlights the need to continue studying the application of EKPR to organic pollutants.

Another important issue that has not yet been studied is the scaling-up of the EKPR processes. In fact, in the field of phytoremediation, mesocosm-scale experiments have been generally used in the research regarding constructed wetlands [21,22], with other few examples of phytostabilization [23,24]. With regard to electrokinetic remediation, our research group have reported several studies focused on the electrokinetic flushing of organic pesticides at large scales [25–28]. Based on the success of the application of EKPR to atrazine-spiked soils using ryegrass (*Lolium perenne* L.) [19], in this work we report the application of EKPR to a low permeability soil spiked with atrazine using a

mesocosm that contained 0.386 m³ of soil planted with ryegrass. This plant species was selected mainly due to its fine and profuse fasciculated root system which can maximize the surface area in contact with the soil; so, it results in an ideal vegetative cover for the in situ treatment of contaminated soils [29]. Another mock-up with the same size but without plants, to which the electric current was also applied, was used as a control treatment. The two mesocosms were divided into ten different soil sections (five cross sections, each one divided into two levels in depth) in which the process parameters were registered in a separated way. The goals of this research were: (i) to describe the changes in some selected soil properties caused by the application of a DC electric field continuously; (ii) to follow the changes in soil pore water characteristics, i.e. pH, electrical conductivity and atrazine residues, throughout the experiment; (iii) to analyse the biomass of ryegrass and the atrazine accumulated in the plant tissues of different soil sections; (iv) to analyse the spatial distribution of the atrazine remaining in the mock-up after the electrokinetic treatments.

2. MATERIALS AND METHODS

2.1. Soil, ryegrass seeds and chemicals

TABLE 1

The tests were carried out using a natural soil coming from a local quarry located near Toledo, central Spain. The absence of atrazine in that soil had been previously checked; it was taken from a soil layer located between 0.15 and 0.5 m depth (for the removal of the vegetal cover). The soil was spread outdoors and air-dried during 2 days with mean day/night temperatures of about 26/7.5°C. Next, the soil was disaggregated, and stones

1 and other strange elements were removed prior to the experiment. The main physical and
2 chemical properties of the soil were analysed by means of normalized methods; they are
3 shown in Table 1. The soil had a low hydraulic conductivity; it was classified as a low
4 plasticity clay (CL) soil according to the Unified Soil Classification Systems (USCS).
5 The acid/base buffer capacity of the soil was determined according to the method
6 described by Yeung et al. [30]; since the addition of acid or base only changed the soil
7 pH in the range 6-11 [18], it can be said that it possesses a very high acid/base buffer
8 capacity. Ryegrass (*Lolium perenne* L.) certified seeds were obtained from the Oregon
9 Seed Certification Service (Corvallis, USA). All seeds were directly sown without any
10 previous pre-germination treatment.

11
12 Atrazine herbicide with analytical standard quality (Fluka, purity 99.1%) was received as
13 a solid from which the aqueous solutions added to the soil were prepared. Acetonitrile
14 HPLC gradient grade (Merck, Germany) was used as solvent for the extraction of solid
15 samples (soil and plant ones). Ultrapure water (Millipore Milli-Q system, resistivity 18.2
16 MΩ cm at 25 °C) was used to prepare all solutions.

17 18 **2.2. Experimental setup**

19
20 **FIGURE 1A**

21
22 **FIGURE 1B**

23
24 Mock-ups used in this work are shown in Figure 1; they were made of glass fibre
25 reinforced PVC with dimensions of 2.25 (L) x 0.49 (W) x 0.50 (H) m. A total volume of

0.386 m³ of soil was placed in each of the two mock-ups that were used in this study, corresponding to EKPR and electrokinetic remediation (EKR) treatments. These two experiments were performed in a potentiostatic mode, i.e., using a voltage gradient of 0.6 V cm⁻¹. The DC electrical field was applied continuously without changing polarity; the electrodes located at the left and right ends of the mock-ups (Figure 1A) worked as anode and cathode, respectively. The duration of the experiment was 19 days.

Each mock-up was loaded with the unpolluted natural soil, which was compacted manually (Figure 1B). Two graphite electrodes (length 0.50 m, diameter 0.15 m) provided by Carbosystem (Madrid, Spain) were directly inserted in the soil at both ends of the mock-ups, with 2 m of separation. They were connected to the power supply (Delta Electronika S.V., model SM120-13, 0-120 V; 0-13 A) with metal clamps.

Soil in the mock-ups was divided (without any physical separation) into ten sections corresponding with five 0.45 m wide cross sections, each of which was divided into two layers in depth (Figure 1A). The depth of the upper and bottom layers were 0.12 and 0.23 m, respectively. Two cylindrical wells made of PVC (0.10 m in diameter and 0.5 m in depth) were located beside the electrodes; they were used to control the water level of the mock-up by means of a window (0.25 cm in depth and 0.10 m in width) perforated in the PVC cylinder and covered by a geotextile layer and a plastic mesh (aperture 1 x 1 cm) in order to avoid the siltation of the wells.

The three central cross sections of the upper layer were sown with ryegrass (22 g of seed in each section) and the plants were initially grown in the unpolluted soil for 35 days. The experiment was carried out in a greenhouse with temperatures varying in the range of

21.6-35.8/ 16.8-21.2 °C day/night. 35 days after planting, the soil was superficially spiked with atrazine at a dose of 2 mg kg⁻¹ soil, corresponding to 1250 mg of atrazine per mock-up (corresponding to 5.80 mmol of atrazine). 12 hours after polluting, DC electric current was switched on. The electric current was recorded daily in each mock-up and the soil temperature was measured in the surface layer of the soil (2-5 cm) by means of a digital probe thermometer (TFA, Germany). Irrigation of the mock-ups was carried out daily by adding tap water in the well located beside the anode; a variable water amount, ranging from 2 to 5 L per day, was added in order to balance the water level of both wells.

Soil pore water samples were taken by means of ten Rhizon samplers located in the centre of each soil section on days 0, 1, 2, 3, 5, 7, 9, 13 and 19 after switching on the electric field (the sample corresponding to the day 0 was taken 2 hours after connecting the power supply). Separated soil samples corresponding to the ten sections of the mock-ups (Figure 1A) were collected at the end of the experiment. Water and soil samples were analysed for pH and ATR residues concentration (atrazine and its metabolites, i.e. deethylatrazine, DEA, and deisopropylatrazine, DIA). Water content was also determined in the soil samples by using a gravimetric method (24 h at 105°C). Finally, plant biomass grown in the three central sections of the planted mock-up (sections 2, 3 and 4, Figure 1A) were taken at the end of the experiment and the dry plant weight was recorded. Ryegrass shoots were harvested by cutting the stem 1 cm above the soil surface. Roots were harvested by carefully separating the biomass from the soil and rinsed thoroughly with deionized water. Plant samples were air-dried for 2 days and homogenized using a grinder (Retsch Model ZM200, Germany) before analysing the concentration of ATR residues in shoots and roots.

2.3. Atrazine residues analysis procedure

Atrazine and its main metabolites (DEA and DIA) were quantified in soil and plant samples by analysing the extracts obtained from a shaking-centrifuging extraction procedure described elsewhere [17,31]. In brief, homogeneous samples of approximately 2 g were extracted with 3 mL of pure acetonitrile in three successive times obtaining an only extract which was analysed by HPLC. Times of shaking and centrifugation corresponding to each extraction step were 30 and 15 min, respectively. Soil and plant extracts and soil pore water samples were filtered with 0.45 µm nylon syringe filters before HPLC analysis.

Atrazine, DEA and DIA were analysed by reversed-phase liquid chromatography using a Shimadzu Prominence UFLC XR (Japan) HPLC system equipped with a diode array detector (SPD-M20A, Shimadzu, Japan) and an analytical C18 reversed-phase column (5 µm, 240 x 4 mm). A mobile phase consisting of a pure acetonitrile (ACN) and 0.01 M phosphate buffer solution (pH=7.1), with a flow rate of 0.5 mL min⁻¹ for 25 min, was used. Temperature oven was kept constant at 30 °C. The HPLC determination was conducted in a linear gradient mode with the following conditions: from 30:70 (v/v) ACN:buffer to 40:60 in 3 min, from 40:60 to 50:50 in 4 min, from 50:50 to 30:70 in 3 min and a final isocratic gradient of 30:70 ACN:buffer for 15 min. The detection wavelength used for all the ATR residues was 222 nm and the retention times were 16.1, 7.9 and 4.5 min for ATR, DEA and DIA, respectively.

3. RESULTS AND DISCUSSION

3.1. Electrical current measurement

FIGURE 2

The electrical current measured in both mock-ups during the experiment is shown in Figure 2. It varied in the ranges of 0.38-0.43 A and 0.31-0.40 A for the planted (EKPR experimental series) and unplanted (EKR experimental series) mesocosms. In the electrokinetic remediation of soils, electric current intensity is a measurement of the soil resistance which is, in turn, controlled by several factors such as moisture, porosity, ionic concentration, temperature and the electrochemical processes occurring in the electrodes. Considering that the extent of the electrochemical reactions should be similar in both mock-ups, the higher values of electric current observed in the EKPR series must be mainly due to the presence of ryegrass plants. In fact, plant roots can increase the porosity of the soil enhancing water and ion transport [17]; moreover, plants increase the ionic concentration of the soil due to the release of root exudates with charged chemical groups [32,33]. A continuous and slight decay of the electric current with time was also observed in both experimental series (Figure 2); it may be related with the decrease in the soil ionic species and other operational processes which increase the electrical resistance of the soil [12,25,34,35].

An expected effect of the electrokinetic remediation is the ohmic heating of the soil [26]. In our experiment, the values of the surface soil temperature measured in the two mock-ups (EKR and EKPR experimental series) were slightly increased as compared to that of the soil arranged in a plastic container (0.075 m³) with no plants and electricity, which was located in the greenhouse and used with comparative purposes (control soil, see

Supplementary Material, Table SM-1). More specifically, surface soil temperature mean values were 26.3°C and 24.7°C for EKPR and EKR series, respectively, vs 23.7°C for the control soil. No significant differences between different soil sections were found. This slight increase in soil surface temperature agrees with previous findings from the electrokinetic remediation of soils polluted by the pesticides 2,4-D and oxyfluorfen in mock-ups with a similar size as those used here [25,36]. On the other hand, the difference of approximately 1-1.5°C between planted and unplanted treatments points out that the presence of a plant cover decreases the heat dissipation from the soil to some extent. Lastly, daily variations of soil temperature shown in Table SM-1 corresponded approximately with the detected variations in the temperature of the greenhouse air (data not shown).

3.2. Soil and soil pore water properties

FIGURE 3

Figure 3 shows the pH values of the different soil sections at the end of the experiment together with the pH changes of the corresponding soil pore water samples throughout the experiment. As it can be seen, soil pH ranged between 7.53 and 9.38 for the EKR series (Fig. 3A and 3C) and between 8.33 and 9.62 for the EKPR series (Fig. 3B and 3D). Soil pH showed a clear pattern in both experimental series, which consisted in increasing pH values from the section 1_A (anode), to the section 5_C (cathode). This trend corresponds to the expected one for an electroremediation process without polarity reversal: acidification and alkalisation in the anode and cathode, respectively. In fact, these variations have been previously reported in previous works of electrokinetic

remediation in mock-ups using facing rows of electrodes [37,38]; they are explained by the electrolysis reactions occurring in the anode and cathode, which generate hydrogen (H^+) and hydroxyl (OH^-) ions, respectively [4]. However, despite not using polarity reversal, the high buffer capacity of the used soil avoided extreme soil pH values and, moreover, the presence of plants led to a narrower pH profile than that of the unplanted mesocosm. It highlights the relevant role of plants in controlling the potential pH changes caused by the electrokinetic treatment, which could negatively affect the soil quality after the remediation. The wider range of pHs throughout the soil profile was found for the bottom layer of the EKR series, i.e. 1.85 units vs 0.89 for the top layer (Fig. 3A and C); this was because the soil from the lower part of the mesocosms was partially saturated of water, with the subsequent improvement on the mobility of ionic species. The higher pH variation in the top layer of the EKPR mock-up as compared to the EKR one, i.e. 1.09 units vs 0.89 (Fig. 3B and D), gives additional evidence about the effect of soil humidity (increased by the plant roots) in the observed pH changes.

pH values corresponding the soil pore water samples taken by Rhizon samplers did not show a pattern as clear as that of soil pH (Fig. 3). In general, pH values of pore water were lower than the corresponding final soil pH with most values ranging between 6 and 9; they also showed an increasing profile from the anode (section 1_A) to the cathode (section 5_C), specially for the last sampling days. Nevertheless, it also can be found pH values much lower or much higher, such as 2.45 (Fig. 3B, section 4, day 9), 5.25 (Fig. 3A, section 1_A, day 9 and Fig. 3B, section 1_A, day 19) or 11.50 (Fig. 3D, section 5_C, day 19). As expected, some of these extreme values were measured in the electrode soil sections. It means that soil water from Rhizon samples was not in equilibrium with the solid phase of the bulk soil. This fact together with the preferential pathways eventually

caused by the Rhizon samplers' suction could explain the absence of a clearer trend in the spatial and temporary changes of the soil pore water pH.

FIGURE 4

Another important parameter in electrokinetic remediation processes is the final water content of the soil, because it is an indirect measurement of the different water fluxes which take place during the remediation [36]. Figure 4 shows the water content of the different soil sections at the end of the test for both experimental series.

In general, EKPR soil moisture values were higher than those of the EKR series, showing that the vegetal cover decreased the water losses by evaporation to a significant extent. The longitudinal profiles of soil water content were different in the two experimental series, especially for the soil sections belonging to the bottom layer. In the unplanted mock-up (EKR series) the soil moisture increased progressively from the anode bottom section to the cathode one; for the top layer, water soil content was also higher in the anode with respect to the cathode but it reached a maximum in the central cross section (section 3) (Figure 4A). Moreover, soil moisture values of the top layer soils sections were lower than those of the bottom layer. It may be explained as a result of two different water fluxes: (i) the electroosmotic flux, which causes the longitudinal transport of the soil pore water from the anode to the cathode, and (ii) the gravity flux, responsible of the vertical water movement [34]. Moreover, it must be considered that the irrigation water was added in the well located in the anode soil section; it would explain the higher water content of section 1_A as compared to section 2. Lastly, the presence of ryegrass plants in the sections 2, 3 and 4 of the planted mock-up (EKPR series) led to longitudinal profiles

reaching the highest soil moisture values in the sections 3 and 4 as a result of the root suction (Figure 4B). Another evidence of the electro-osmotic flux was the daily observed higher water level in the well located near the cathode respect to that of the anode (around 0.5 cm, data not shown).

FIGURE 5

Figure 5 shows the changes in the electrical conductivity (EC) of the soil pore water samples together with the soil EC at the end of the experiment. Soil EC decreased from the anode section to the cathode one (except for the section 3 in the top layer of the EKR mock-up, Figure 5A) and it was higher for the upper soil layer than for the bottom one. This trend is just the opposite to that observed for the soil pH (Figure 3) and it can be explained considering that the EC of the hydrogen ions is quite higher than that of hydroxyl ions (35.0 vs $19.8 \text{ mS m}^2 \text{ mol}^{-1}$) and the other soil anions (chloride, sulphate, nitrate, etc.) [39]. However, the spatial variation of the soil pore water EC was some different. On one hand, this parameter was higher for the samples belonging to the bottom layer of both mock-ups. On the other hand, in the EKR mesocosm, it clearly decreased from the anode to cathode soil sections, while this trend was not found or was slighter for the EKPR one. The vertical variation in water EC can be explained in terms of the higher water content of the bottom layer which led to a higher mobilization of soil ions. The longitudinal variation observed in the EKR mock-up would be related with the mentioned higher EC of the H^+ ions, while in the planted mock-up, water EC would be strongly influenced by both the release of ionic root exudates and the water suction by plant roots. Regarding the temporary changes in soil pore water EC, with few exceptions, it increased in the first days of the experiment and, after that, decreased until the end of the test. The

1 initial increase in EC was due, on one hand, to the generation of hydrogen and hydroxyl
2 ions in the electrode sections and, on the other hand, to the movement of those ions and
3 the others from soil caused by the electro-osmosis and electromigration fluxes. As
4 experiment progressed, the neutralization of the acid and basic fronts and the precipitation
5 of certain ions will gradually reduce the water EC [5,27]. It agrees with the decrease on
6 electric current with time described in the Section 3.1.

8 **3.3. Distribution of atrazine residues in soil pore water**

10 **FIGURE 6**

12 The concentration of atrazine residues (atrazine, ATR, and its main metabolites,
13 deethylatrazine, DEA, and deisopropylatrazine, DIA) in the soil water solution from the
14 different soil sections was monitored in order to determine the main transport mechanisms
15 involved in the studied EKPR process. Figure 6 shows the concentration of atrazine
16 residues ($\mu\text{mol L}^{-1}$) taken by the Rhizon samplers located in the centre of the ten soil
17 sections in which the mock-ups were divided (Figure 1A). As it was described in Section
18 2.2, the soil was spiked superficially and homogeneously with an aqueous solution of
19 atrazine and 12 hours later, the power supply was connected; the first sampling of soil
20 pore water was carried out two hours after switching on the electric field (day 0).

22 Firstly, the concentration of ATR residues in both experimental series was much higher,
23 in approximately one order of magnitude, in the water samples from the top layer sections
24 than in the bottom layer ones (Figure 6). It means that atrazine was effectively mobilized
25 by the electric field in both mock-ups with a predominance of the horizontal flux

(electrokinetic) vs the vertical one (gravity). The transport mechanisms of atrazine residues in soils subjected to an electric field are electroosmosis and electromigration [17,40]. Electromigration, that is, the transport of negatively charged species of atrazine to the anode, is possible because the pK_a values of ATR, DEA and DIA (in the range of 1.50-1.71, Table SM-2), which cause the ionic atrazine species to be the predominant ones in moderately acid to alkaline media (soil or pore water) [41]; so, the transport of charged ATR species by electromigration could also contribute to increase soil water EC near the anode. The other electrokinetic flux, i.e. electro-osmosis, is the transport of water molecules to the cathode as a consequence of the migration of soil positive ions under an electric potential [5]; moderately polar molecules, such as ATR, DEA and DIA, may be transported by electro-osmosis as dissolved aqueous species. Both transport mechanisms, with opposite direction, caused that atrazine residues were mostly accumulated in the sections 1_A and 5_C of the top layer (Figures 6A and 6C) from the first hours after the electric current was connected. It does not mean that atrazine residues were only located in these soil sections, but only in the electrode sections they reached an enough concentration to be detected in significant concentrations in the aqueous phase (5-60 $\mu\text{mol L}^{-1}$). As the experiment progressed, the concentration of ATR residues in the soil pore water decreased in the top layer sections (Figures 6A and 6C). For the EKR series, ATR residues concentration reached values below 5 $\mu\text{mol L}^{-1}$ from day 3 to day 7 for the anode and cathode sections, respectively (Figure 6A); for the EKPR series, those values were reached from days 7 and 5, respectively (Figure 6C). It was mainly due to the removal of atrazine from the soil by different mechanisms which will be discussed in the Section 3.5. Regarding the soil sections of the bottom layer, significant amounts of ATR residues ($> 1 \mu\text{mol L}^{-1}$) were detected in the anode section of the planted mock-up from the first hours after starting the experiment (Figure 6B) and from the day 5 in the cathode section of both

experimental series (Figures 6B and 6D). DEA and DIA metabolites were the atrazine species predominant in the soil pore water samples corresponding to the last samplings (days 13 and 19), especially for the bottom layer soil sections of the planted mock-up; their concentration in the top and bottom layer samples were 50.9% and 59.0% of the total ATR residues, respectively, for the EKR series mock-up and 57.2% and 77.3% for the EKPR series mock-up.

It is remarkable that the presence of ryegrass plants led to significant higher concentrations of ATR residues in the left soil sections (1_A and 2) of the planted mock-up as compared to those of the unplanted one. It means that ryegrass avoided electro-osmotic flux of atrazine to some extent since water suction by roots could reasonably affect the movement of water from anode to cathode. So, electromigration flux of atrazine was more relevant in the EKPR process. Together with the electrokinetic fluxes, the gravity flux also contributed to the vertical dispersion of the atrazine residues in the soil, especially from half the duration of the experiment. Lastly, it may be pointed out that the ideas presented here for the atrazine transport processes are in good agreement with the profiles of soil water content discussed in the previous section.

3.4. Biomass production and ATR residues accumulated in plant tissues

TABLE 2

The values of harvested biomass (root and shoot, g) at the end of the experiment are shown in Table 2 together with the concentration of atrazine and its metabolites (DEA+DIA) in the ryegrass tissues. Plant biomass production was similar for the three

1 planted soil sections (2, 3 and 4) with a slightly higher value for the middle section (3). It
2 must be considered that ryegrass plants were grown during 5 weeks before atrazine
3 spiking and the application of the electric current; as a result, changes in the properties of
4 the different soil sections occurred during the 19 days that lasted the EKPR test had not a
5 relevant effect on plant growth. Nevertheless, it has previously reported that both atrazine
6 and electric field cause negative and crossed effects on ryegrass biomass [10,14,19,42].
7

8 Total plant ATR concentrations were in the range of 321.66-461.30 $\mu\text{mol kg}^{-1}$ and
9 followed this trend: section 4 > section 2 > section 3. Concentrations of ATR metabolites
10 were quite lower (23.19-26.63 $\mu\text{mol kg}^{-1}$) and very similar for all the soil sections. It
11 closely agrees with the distribution of atrazine residues in the soil pore water in the first
12 days of the EKPR experiment (See Section 3.3) and highlights the fact that the electric
13 current effectively increases plant uptake by increasing the mobility of pollutants in soils.
14 Moreover, it can be suggested that the higher biomass of ryegrass in the middle section
15 (3) was related with the lower atrazine accumulation. The order of magnitude of ATR
16 residues plant concentrations was the same as that previously found by us in an EKPR
17 pot experiment using ryegrass [19] although the ratio $\text{ATR}_{\text{shoots}} / \text{ATR}_{\text{roots}}$ (translocation
18 factor) were significantly lower in this case (1.3-2.8 vs 11.0-68.7). The latter can be due
19 to a higher soil atrazine availability until the end of the test (demonstrated by the fact that
20 the overall atrazine soil removal was significantly lower in the present experiment, see
21 Section 3.5); therefore, atrazine plant uptake took place during the entire duration of the
22 experiment keeping a subsequent relatively high ATR concentration in roots. On the
23 opposite, the much higher ratio of ATR metabolites in shoots than roots may be due to
24 that atrazine degradation mainly takes place in the aerial tissues of ryegrass and/or ATR

metabolites are more efficiently translocated to shoots because of their higher water solubility (Table SM-2).

3.5. ATR residues remaining in soil and overall removal efficiency

FIGURE 7

Atrazine residues remaining in the different soil sections of both mock-ups at the end of the experiment are shown in Figure 7. It can be observed that most of the atrazine remaining in the soil was accumulated in the cathode section (5_C), reaching values up to four times higher than those of the other sections for the top layer (in both experimental series) and approximately two-fold for the bottom layer (only for the planted mock-up). These results complement the soil pore water data discussed in the Section 3.3 and point out the predominant role of the electro-osmotic transport of atrazine in the studied electrokinetic treatment process. Moreover, this fact strongly agrees with our previous findings in the study of EKPR of atrazine by maize; in that case, electro-osmosis was the main mechanism of atrazine residues transport when an electric field of 2 V cm^{-1} with polarity reversal was applied [18]. The anomalous higher ATR residues accumulation in the bottom layer section 4 of the unplanted mock-up (Figure 7B) may be explained as a result of the opposite directions of the electro-osmotic and electromigration fluxes of atrazine and its hydrolysis products. The fact that ATR residues concentrations are much higher for the top layer soil sections than for the bottom layer ones (up to one order of magnitude) reinforces the hypothesis of the greater importance of the horizontal (electrokinetic) flow over the vertical (by gravity) one and agrees with what was observed for atrazine residues concentration in soil pore water (Section 3.3).

TABLE 3

The essential role of plants in the atrazine removal from soils by the EKPR technology is remarked by the fact that atrazine residues concentrations of the planted soil sections (2, 3 and 4) were the lowest for the EKPR series mock-up and significantly lower than those corresponding to the EKR series mock-up (Figure 7). However, since ATR residues accumulated in ryegrass tissues only accounted for 2.03% of the initial atrazine (1.91% as parent ATR and 0.12% as ATR metabolites, Table 3), it does not seem that plant accumulation is the main mechanism of ATR removal from soils. The detection of important amounts of the deethylatrazine and deisopropylatrazine in both plant tissues and soil (Figure 7 and Table 3) is indicative of a significant biochemical atrazine degradation, conducted by plants and rhizosphere microorganisms, took place in the studied process. So, phytoextraction, phytodegradation and rhizodegradation mechanisms seem to contribute together to the atrazine removal in electrokinetic-assisted phytoremediation of soils. Some minor losses of atrazine by volatilization could be expected because the water evaporation from the upper soil layer. Microbial degradation seems to be the main mechanism of atrazine removal in the EKR series mock-up; in fact, DEA and DIA were also found in the soil from the unplanted mock-up (Figure 7 and Table 3), which was not sterilized before its use. The values of the ratio (DEA+DIA)/ATR soil concentrations were higher in the planted mock-up as compared to those of the unplanted one, 1.97 vs 0.91, respectively (Table 3); it means that plant roots increased biological degradation some extent because they affect the structure and the activity of microbial communities near the roots [43,44].

Table 3 shows the overall ATR removal after 19 days for both mock-ups considering the initial atrazine added to the soil. Atrazine metabolites remaining in the soil are also shown in Table 3, but they have not been taken into account for this calculation because DEA and DIA are considered less toxic than the parent atrazine [45]. For the unplanted mock-up the percentage of atrazine removal was 40.20% while it was improved to 61.01% when ryegrass was used in combination with electrokinetic remediation. These results differ from those obtained by us in an EKPR pot experiment carried out using ryegrass and an electric field of 1 V cm^{-1} applied in a continuous mode; ATR removal values were 94.45 and 84.32% for ryegrass and unplanted pots, respectively [19]. Two issues can be considered in order to explain this difference. Firstly, the pot experiment was conducted using periodical changes of electrical polarity (each 24 h); secondly, the percentage of the soil in contact with ryegrass roots was much higher for the pot experiment (0.2 g of roots per kg of soil in the mock-up vs 1.5 g of roots per kg of soil in the pots). Both aspects contribute enhancing the availability of atrazine for ryegrass roots and microorganisms improving phyto- and biodegradation processes. Nevertheless, comparisons between the results of soil electrokinetic remediation from different scales must be carefully considered because it has been previously shown that the application of the same voltage gradient does not guarantee a similar extent in the development of the electrokinetic parameters [46].

Lastly, although the experimental spiking procedure used here ensured a homogeneous distribution of atrazine on the soil surface, from our results it seems that atrazine was not well dispersed through the entire soil volume of the mock-ups at the beginning of the EKPR test. Later, when the electric field was switched on, atrazine distribution was strongly conditioned by the electrokinetic fluxes. Therefore, it may be expected a very

different performance in the application of EKPR to real field conditions due to the previous aging of atrazine pollution and the different extent of the electro-osmotic, electromigration and vertical transport mechanisms. So, it is strongly recommended to carry out deeper studies focused on the scaling up of electrokinetic-assisted phytoremediation before its application to polluted soils in real conditions.

4. CONCLUSIONS

From this work, the following conclusions can be drawn:

- The application of the electric field to the soil of the mock-ups led to a moderately increasing pH soil profile from the anode to the cathode, due to the water electrolysis reactions taking place; soil pore water pH followed a similar pattern than those of soil but showing some punctual extreme values.
- Ryegrass biomass was similar in the three planted soil sections of the planted mock-up although it was slightly influenced by the atrazine plant accumulation.
- Concentration values of atrazine residues remaining in the different soil sections of both mock-ups at the end of the experiment were in good agreement with those of the soil pore water data, with most of the atrazine being accumulated in the cathode section.
- Atrazine was mainly removed from soils by biochemical degradation, as suggested by the relevant presence of the metabolites DEA and DIA in soil and plant tissues.

- Atrazine removal was greatly improved by ryegrass plants leading to an overall increase from 40.20% (unplanted mock-up) to 61.01% (planted mock-up).

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REFERENCES

- [1] A. Ene, O. Bogdevich, A. Sion, Levels and distribution of organochlorine pesticides (OCPs) and polycyclic aromatic hydrocarbons (PAHs) in topsoils from SE Romania, Sci. Total Environ. 439 (2012) 76–86. doi:10.1016/j.scitotenv.2012.09.004.
- [2] C. Cameselle, R.A. Chirakkara, K.R. Reddy, Electrokinetic-enhanced phytoremediation of soils: Status and opportunities, Chemosphere. 93 (2013). doi:10.1016/j.chemosphere.2013.06.029.
- [3] Y.B. Acar, A.N. Alshawabkeh, Principles of Electrokinetic Remediation, Environ. Sci. Technol. 27 (1993) 2638–2647. doi:10.1021/es00049a002.

- [4] J. Virkutyte, M. Sillanpää, P. Latostenmaa, Electrokinetic soil remediation - Critical overview, *Sci. Total Environ.* 289 (2002) 97–121. doi:10.1016/S0048-9697(01)01027-0.
- [5] K.R. Reddy, C. Cameselle, *Electrochemical Remediation Technologies for Polluted Soils, Sediments and Groundwater*, 2009. doi:10.1002/9780470523650.
- [6] A. Genc, G. Chase, A. Foos, Electrokinetic removal of manganese from river sediment, *Water. Air. Soil Pollut.* 197 (2009) 131–141. doi:10.1007/s11270-008-9796-7.
- [7] A.L. Salido, K.L. Hasty, J.-M. Lim, D.J. Butcher, Phytoremediation of arsenic and lead in contaminated soil using Chinese Brake ferns (*Pteris vittata*) and Indian mustard (*Brassica juncea*), *Int. J. Phytoremediation.* 5 (2003) 89–103. doi:10.1080/713610173.
- [8] B. V Tangahu, S.R. Sheikh Abdullah, H. Basri, M. Idris, N. Anuar, M. Mukhlisin, A review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation, *Int. J. Chem. Eng.* (2011). doi:10.1155/2011/939161.
- [9] J.M. Lim, A.L. Salido, D.J. Butcher, Phytoremediation of lead using Indian mustard (*Brassica juncea*) with EDTA and electrodics, *Microchem. J.* 76 (2004) 3–9. doi:10.1016/j.microc.2003.10.002.
- [10] D.M. Zhou, H.F. Chen, L. Cang, Y.J. Wang, Ryegrass uptake of soil Cu/Zn induced by EDTA/EDDS together with a vertical direct-current electrical field, *Chemosphere.* 67 (2007) 1671–1676. doi:10.1016/j.chemosphere.2006.11.042.
- [11] R. Bi, M. Schlaak, E. Siefert, R. Lord, H. Connolly, Influence of electrical fields (AC and DC) on phytoremediation of metal polluted soils with rapeseed (*Brassica napus*) and tobacco (*Nicotiana tabacum*), *Chemosphere.* 83 (2011) 318–326. doi:10.1016/j.chemosphere.2010.12.052.

- [12] L. Cang, Q.Y. Wang, D.M. Zhou, H. Xu, Effects of electrokinetic-assisted phytoremediation of a multiple-metal contaminated soil on soil metal bioavailability and uptake by Indian mustard, *Sep. Purif. Technol.* 79 (2011) 246–253. doi:10.1016/j.seppur.2011.02.016.
- [13] L. Cang, D.M. Zhou, Q.Y. Wang, G.P. Fan, Impact of electrokinetic-assisted phytoremediation of heavy metal contaminated soil on its physicochemical properties, enzymatic and microbial activities, *Electrochim. Acta.* 86 (2012) 41–48. doi:10.1016/j.electacta.2012.04.112.
- [14] C.S. O'Connor, N.W. Lepp, R. Edwards, G. Sunderland, The combined use of electrokinetic remediation and phytoremediation to decontaminate metal-polluted soils: A laboratory-scale feasibility study, *Environ. Monit. Assess.* 84 (2003) 141–158. doi:10.1023/A:1022851501118.
- [15] R.A. Chirakkara, K.R. Reddy, C. Cameselle, Electrokinetic Amendment in Phytoremediation of Mixed Contaminated Soil, *Electrochim. Acta.* 181 (2015) 179–191. doi:10.1016/j.electacta.2015.01.025.
- [16] G. Acosta-Santoyo, C. Cameselle, E. Bustos, Electrokinetic – Enhanced ryegrass cultures in soils polluted with organic and inorganic compounds, *Environ. Res.* 158 (2017). doi:10.1016/j.envres.2017.06.004.
- [17] V. Sánchez, F.J. López-Bellido, P. Cañizares, L. Rodríguez, Can electrochemistry enhance the removal of organic pollutants by phytoremediation?, *J. Environ. Manage.* 225 (2018) 280–287. doi:10.1016/j.jenvman.2018.07.086.
- [18] V. Sánchez, F.J. López-Bellido, M.A. Rodrigo, L. Rodríguez, Electrokinetic-assisted phytoremediation of atrazine: Differences between electrode and interelectrode soil sections, *Sep. Purif. Technol.* 211 (2019) 19–27. doi:10.1016/j.seppur.2018.09.064.

- 1 [19] V. Sánchez, Francisco, J. López-Bellido, M.A. Rodrigo, L. Rodríguez, Enhancing
2 the removal of atrazine from soils by electrokinetic-assisted phytoremediation
3 using ryegrass (*Lolium perenne* L.), *Chemosphere*. (2019).
4 doi:<https://doi.org/10.1016/j.chemosphere.2019.05.216>.
- 5 [20] I.M. V Rocha, K.N.O. Silva, D.R. Silva, C.A. Martínez-Huitle, E. V Santos,
6 Coupling electrokinetic remediation with phytoremediation for depolluting soil
7 with petroleum and the use of electrochemical technologies for treating the effluent
8 generated, *Sep. Purif. Technol.* 208 (2019) 194–200.
9 doi:[10.1016/j.seppur.2018.03.012](https://doi.org/10.1016/j.seppur.2018.03.012).
- 10 [21] Q. Yan, Y. Xu, Y. Yu, Z.W. Zhu, G. Feng, Effects of pharmaceuticals on microbial
11 communities and activity of soil enzymes in mesocosm-scale constructed
12 wetlands, *Chemosphere*. 212 (2018) 245–253.
13 doi:[10.1016/j.chemosphere.2018.08.059](https://doi.org/10.1016/j.chemosphere.2018.08.059).
- 14 [22] J. Villaseñor Camacho, A. De Lucas Martínez, R. Gómez Gómez, J. Mena Sanz,
15 A comparative study of five horizontal subsurface flow constructed wetlands using
16 different plant species for domestic wastewater treatment, *Environ. Technol.* 28
17 (2007) 1333–1343. doi:[10.1080/09593332808618897](https://doi.org/10.1080/09593332808618897).
- 18 [23] A. Valentín-Vargas, J.W. Neilson, R.A. Root, J. Chorover, R.M. Maier, Treatment
19 impacts on temporal microbial community dynamics during phytostabilization of
20 acid-generating mine tailings in semiarid regions, *Sci. Total Environ.* 618 (2018)
21 357–368. doi:[10.1016/J.SCITOTENV.2017.11.010](https://doi.org/10.1016/J.SCITOTENV.2017.11.010).
- 22 [24] A. Ruttens, J. V Colpaert, M. Mench, J. Boisson, R. Carleer, J. Vangronsveld,
23 Phytostabilization of a metal contaminated sandy soil. II: Influence of compost
24 and/or inorganic metal immobilizing soil amendments on metal leaching, *Environ.*
25 *Pollut.* 144 (2006) 533–539. doi:[10.1016/j.envpol.2006.01.021](https://doi.org/10.1016/j.envpol.2006.01.021).

- [25] C. Risco, S. Rodrigo, R. López Vizcaíno, A. Yustres, C. Saez, P. Cañizares, V. Navarro, M.A. Rodrigo, Removal of oxyfluorfen from spiked soils using electrokinetic soil flushing with linear rows of electrodes, *Chem. Eng. J.* 294 (2016) 65–72. doi:10.1016/j.cej.2016.02.112.
- [26] C. Risco, H. Rubí-Juárez, S. Rodrigo, R. López Vizcaíno, C. Saez, P. Cañizares, C. Barrera-Díaz, V. Navarro, M.A. Rodrigo, Removal of oxyfluorfen from spiked soils using electrokinetic fences, *Sep. Purif. Technol.* 167 (2016) 55–62. doi:10.1016/j.seppur.2016.04.050.
- [27] R. López-Vizcaíno, C. Risco, J. Isidro, S. Rodrigo, C. Saez, P. Cañizares, V. Navarro, M.A. Rodrigo, Scale-up of the electrokinetic fence technology for the removal of pesticides. Part I: Some notes about the transport of inorganic species, *Chemosphere.* (2017). doi:10.1016/j.chemosphere.2016.09.113.
- [28] R. López-Vizcaíno, C. Risco, J. Isidro, S. Rodrigo, C. Saez, P. Cañizares, V. Navarro, M.A. Rodrigo, Scale-up of the electrokinetic fence technology for the removal of pesticides. Part II: Does size matter for removal of herbicides?, *Chemosphere.* (2017). doi:10.1016/j.chemosphere.2016.09.114.
- [29] W. Aprill, R.C. Sims, Evaluation of the use of prairie grasses for stimulating polycyclic aromatic hydrocarbon treatment in soil, *Chemosphere.* 20 (1990) 253–265. doi:10.1016/0045-6535(90)90100-8.
- [30] A.T. Yeung, C.-N. Hsu, R.M. Menon, EDTA-enhanced electrokinetic extraction of lead, *J. Geotech. Eng.* 122 (1996) 666–673. <https://www.scopus.com/inward/record.uri?eid=2-s2.0-0030317705&partnerID=40&md5=674a3244c438c9100779cf940b7544c3>.
- [31] M.F. Amadori, G.A. Cordeiro, C.C. Rebouças, P.G. Peralta-Zamora, M.T. Grassi, G. Abate, Extraction method for the determination of atrazine, deethylatrazine, and

deisopropylatrazine in agricultural soil using factorial design, J. Braz. Chem. Soc.
24 (2013) 483–491. doi:10.5935/0103-5053.20130058.

[32] A. Vassilev, J.P. Schwitzguebel, T. Thewys, D. Van Der Lelie, J. Vangronsveld,
The use of plants for remediation of metal-contaminated soils.,
ScientificWorldJournal. 4 (2004) 9–34. doi:10.1100/tsw.2004.2.

[33] G.H. Lefevre, R.M. Hozalski, P.J. Novak, Root exudate enhanced contaminant
desorption: An abiotic contribution to the rhizosphere effect, Environ. Sci.
Technol. 47 (2013) 11545–11553. doi:10.1021/es402446v.

[34] C. Risco, S. Rodrigo, R. López-Vizcaíno, A. Yustres, C. Sáez, P. Cañizares, V.
Navarro, M.A. Rodrigo, Electrochemically assisted fences for the
electroremediation of soils polluted with 2,4-D: A case study in a pilot plant, Sep.
Purif. Technol. 156 (2015) 234–241. doi:10.1016/J.SEPPUR.2015.10.006.

[35] R.A. Chirakkara, K.R. Reddy, C. Cameselle, Electrokinetic Amendment in
Phytoremediation of Mixed Contaminated Soil, Electrochim. Acta. 181 (2015).
doi:10.1016/j.electacta.2015.01.025.

[36] C. Risco, R. López-Vizcaíno, C. Sáez, A. Yustres, P. Cañizares, V. Navarro, M.A.
Rodrigo, Remediation of soils polluted with 2,4-D by electrokinetic soil flushing
with facing rows of electrodes: A case study in a pilot plant, Chem. Eng. J. (2016).
doi:10.1016/j.cej.2015.10.011.

[37] R. López-Vizcaíno, J. Alonso, P. Cañizares, M.J. León, V. Navarro, M.A. Rodrigo,
C. Sáez, Electroremediation of a natural soil polluted with phenanthrene in a pilot
plant, J. Hazard. Mater. (2014). doi:10.1016/j.jhazmat.2013.11.048.

[38] R. López-Vizcaíno, J. Alonso, P. Cañizares, M.J. León, V. Navarro, M.A. Rodrigo,
C. Sáez, Removal of phenanthrene from synthetic kaolin soils by eletrokinetic soil
flushing, Sep. Purif. Technol. (2014). doi:10.1016/j.seppur.2014.05.004.

- 1 [39] C. Cameselle, S. Gouveia, S. Urréjola, Benefits of phytoremediation amended with
2 DC electric field. Application to soils contaminated with heavy metals,
3 Chemosphere. (2019). doi:10.1016/j.chemosphere.2019.04.222.
- 4 [40] A.T. Yeung, Y.Y. Gu, A review on techniques to enhance electrochemical
5 remediation of contaminated soils, J. Hazard. Mater. 195 (2011) 11–29.
6 doi:10.1016/j.jhazmat.2011.08.047.
- 7 [41] S. Ahmed, M.G. Rasul, R. Brown, M.A. Hashib, Influence of parameters on the
8 heterogeneous photocatalytic degradation of pesticides and phenolic contaminants
9 in wastewater: A short review, J. Environ. Manage. (2011).
10 doi:10.1016/j.jenvman.2010.08.028.
- 11 [42] C. Fang, M. Radosevich, J.J. Fuhrmann, Atrazine and phenanthrene degradation in
12 grass rhizosphere soil, Soil Biol. Biochem. 33 (2001) 671–678.
13 doi:10.1016/S0038-0717(00)00216-9.
- 14 [43] Q. Chaudhry, P. Schröder, D. Werck-Reichhart, W. Grajek, R. Marecik, Prospects
15 and limitations of phytoremediation for the removal of persistent pesticides in the
16 environment, Environ. Sci. Pollut. Res. 9 (2002) 4–17. doi:10.1007/BF02987313.
- 17 [44] S. Piutti, S. Hallet, S. Rousseaux, L. Philippot, G. Soulas, F. Martin-Laurent,
18 Accelerated mineralisation of atrazine in maize rhizosphere soil, Biol. Fertil. Soils.
19 36 (2002) 434–441. doi:10.1007/s00374-002-0545-6.
- 20 [45] X. Fan, F. Song, Bioremediation of atrazine: recent advances and promises, J. Soils
21 Sediments. 14 (2014) 1727–1737. doi:10.1007/s11368-014-0921-5.
- 22 [46] R. López-Vizcaíno, V. Navarro, M.J. León, C. Risco, M.A. Rodrigo, C. Sáez, P.
23 Cañizares, Scale-up on electrokinetic remediation: Engineering and technological
24 parameters, J. Hazard. Mater. (2016). doi:10.1016/j.jhazmat.2016.05.012.

Table 1. Main physical and chemical properties of the soil used in this experiment.

Soil property	Method	Value
pH	1:5 soil/water mixture	9.42
Electrical conductivity ($\mu\text{S cm}^{-1}$)	1:5 soil/water mixture	150
CEC (cmol kg^{-1})	Ammonium acetate saturation method	23.41
Soil organic carbon content (%)	Total organic carbon analyser	0.60
Bulk density (kg m^{-3})	ASTM Standards D-854	1,540
Liquid limit (%)		42
Plastic limit (%)	ASTM Standards D-4318	24
Plasticity index (%)		18
Soil classification	USCS	CL (a low plasticity clay)

Table 2. Plant biomass and concentration of atrazine (ATR) and its metabolites (DEA: deethylatrazine; DIA: deisopropylatrazine) in the ryegrass tissues for the EKPR experimental series.

Soil section	Biomass*		ATR concentration ($\mu\text{mol kg}^{-1}$)			(DEA+DIA) concentration ($\mu\text{mol kg}^{-1}$)		
	Root (g)	Shoot (g)	Root	Shoot	Total plant	Root	Shoot	Total plant
2	40.38	52.70	307.38	461.76	394.79	3.20	38.51	23.19
3	41.77	54.54	159.00	446.24	321.66	2.81	39.53	23.60
4	40.49	52.87	390.85	515.26	461.30	5.23	43.03	26.63

(*) Soil sections 2, 3 and 4 had the same surface area (2,205 cm²)

Table 3. Atrazine (ATR) and its metabolites (DEA: deethylatrazine; DIA: deisopropylatrazine) remaining in soil and accumulated in plants at the end of the experiment (expressed as % of the initial ATR), (DEA+DIA)/ATR ratio and ATR removal from soils (expressed as % of the initial ATR).

Experimental Series*	EKR series	EKPR series
ATR remaining in soil	59.79	38.98
(DEA+DIA) remaining in soil	11.11	9.80
(DEA+DIA)/ATR soil	0.91	1.97
ATR in total plant	-	1.91
(DEA+DIA) in total plant	-	0.12
Overall ATR removal	40.21	61.01

(*) EKR: electrokinetic remediation

EKPR: electrokinetic-assisted phytoremediation

FIGURE CAPTIONS

Figure 1. A) Diagram of the experimental mock-ups, including sampling points for Rhizon samplers (dimensions in meters). B) Pictures of mock-up preparation.

Figure 2. Time course of the electric current for the EKR and EKPR experimental series.

Figure 3. pH of soil pore water Rhizon samples over time and soil pH at the end of the experiment. Values from the different soil sections.

Figure 4. Soil water content profile of the mock-ups (% in mass) corresponding to EKR and EKPR experimental series.

Figure 5. Electrical conductivity (EC) of soil pore water over time and soil EC at the end of the experiment. Values from the different soil sections.

Figure 6. Spatial distribution and time course of atrazine residues (ATR+DEA+DIA) concentration in the soil pore water ($\mu\text{mol L}^{-1}$) (ATR: atrazine, DEA: deethylatrazine, DIA: deisopropylatrazine). Time axis: 0 days corresponds to 2 hours after applying the electric field.

Figure 7. Atrazine (ATR) and its metabolites (DEA: deethylatrazine; DIA: deisopropylatrazine) concentration ($\mu\text{mol kg}^{-1}$ soil) in the different soil sections at the end of the experiment. A: Soil top layer and B: Soil bottom layer.

FIGURE 1A

FIGURE 1B

**(1)
Materials**

**(2)
Placing the first soil layer**

**(3)
Placing rhizon samplers**

**(4)
Seeds germination**

**(5)
Plant growth**

**(6)
Sampling water soil by rhizon samplers**

FIGURE 2

FIGURE 3

FIGURE 4

FIGURE 5

FIGURE 6

FIGURE 7

SUPPLEMENTARY MATERIAL

A mesocosm study of electrokinetic-assisted phytoremediation of atrazine-polluted soils

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Table SM-1. Time course of the surface soil temperature during the entire experiment.

		Time (days)																			
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
RYEGRASS mock-up (EKPR series)																					
Soil sections	1_Anode	26.6	28.3	24.8	23.8	24.7	26.8	25.2	27.6	25.3	24.5	25.1	25.3	25.5	25.5	25.2	25.1	25.1	24.9	25.0	25.2
	2	25.3	27.4	26.1	24.5	26.0	27.6	26.4	27.1	26.6	25.8	26.0	26.5	27.8	27.1	26.3	26.2	26.5	26.5	26.4	26.4
	3	24.5	25.8	25.8	24.9	26.3	27.6	27.2	27.2	27.0	26.2	27.0	27.3	28.4	27.6	26.8	26.8	26.8	27.1	26.6	26.6
	4	24.5	25.8	26.2	25.4	26.4	27.7	27.2	27.8	27.4	26.8	27.0	27.6	28.6	27.8	26.8	26.8	27.5	27.2	27.2	27.0
	5_Cathode	25.5	25.3	25.1	24.5	25.7	26.8	26.4	26.6	25.8	25.0	26.5	26.7	27.1	26.1	26.2	25.6	26.2	26.6	25.5	25.8
Mean		25.3	26.5	25.6	24.6	25.8	27.3	26.5	27.3	26.4	25.7	26.3	26.7	27.5	26.8	26.3	26.1	26.4	26.5	26.1	26.2
(STD)		(0.9)	(1.3)	(0.6)	(0.6)	(0.7)	(0.5)	(0.8)	(0.5)	(0.9)	(0.9)	(0.8)	(0.9)	(1.2)	(1.0)	(0.6)	(0.7)	(0.9)	(0.9)	(0.9)	(0.7)
UNPLANTED mock-up (EKR series)																					
Soil sections	1_Anode	25.1	25.5	24.7	23.9	24.5	26.0	24.8	25.8	23.9	23.6	25.0	25.2	25.9	24.6	24.7	25.1	24.8	25.0	24.3	24.2
	2	24.5	24.8	24.3	23.3	24.3	25.3	24.3	26.3	23.3	23.8	24.5	24.5	24.2	24.1	24.2	24.3	24.2	24.5	24.3	24.2
	3	24.6	24.8	24.6	23.8	24.5	25.6	24.9	25.3	24.2	24.0	25.6	25.2	24.5	24.7	24.8	25.1	25.1	25.0	24.0	24.0
	4	24.9	24.5	24.4	23.6	24.5	25.5	24.8	25.4	24.5	23.9	25.1	25.0	24.9	24.5	25.0	24.8	25.0	25.5	24.2	24.3
	5_Cathode	24.9	24.5	24.3	23.6	24.8	25.8	25.3	25.3	24.8	23.9	25.3	25.3	25.5	24.5	25.1	25.0	24.8	25.3	24.6	24.5
Mean		24.8	24.8	24.5	23.6	24.5	25.6	24.8	25.4	24.1	23.8	25.1	25.0	25.0	24.5	24.8	24.9	24.8	25.1	24.3	24.2
(STD)		(0.2)	(0.4)	(0.2)	(0.2)	(0.2)	(0.3)	(0.4)	(0.2)	(0.6)	(0.1)	(0.4)	(0.3)	(0.7)	(0.2)	(0.3)	(0.3)	(0.3)	(0.4)	(0.2)	(0.2)
CONTROL SOIL																					
Mean		25.0	23.4	23.2	22.8	23.5	23.7	23.5	23.8	23.3	22.9	23.8	24.0	24.3	24.0	23.6	23.8	23.8	23.9	23.2	23.8

Table SM-2. Chemical structure and main properties of atrazine and the metabolites studied in this work (taken from Amadori et al. 2016*).

Name		Key properties	Formula
ATR metabolites	Atrazine (ATR)	215.68 g mol ⁻¹ 33 mg L ⁻¹ (water) pK _a 1.68-1.71
	Deethylatrazine (DEA)	187.63 g mol ⁻¹ 3200 mg L ⁻¹ (water) pK _a 1.30-1.65
	Deisopropylatrazine (DIA)	173.60 g mol ⁻¹ 670 mg L ⁻¹ (water) pK _a 1.30-1.58

(*) Amadori, M.F., Cordeiro, G.A., Rebouças, C.C., Peralta-Zamora, P.G., Grassi, M.T., Abate, G., 2013. Extraction method for the determination of atrazine, deethylatrazine, and deisopropylatrazine in agricultural soil using factorial design. J. Braz. Chem. Soc. 24, 483-491.