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**ENHANCING THE REMOVAL OF ATRAZINE FROM SOILS BY
ELECTROKINETIC-ASSISTED PHYTOREMEDIATION USING RYEGRASS
(*Lolium perenne* L.)**

**Virtudes Sánchez¹, Francisco¹ Javier López-Bellido², Manuel A. Rodrigo¹ and Luis
Rodríguez^{1,*}**

¹ Department of Chemical Engineering, University of Castilla-La Mancha, Avenida
Camilo José Cela, s/n, 13071 Ciudad Real (SPAIN)

² School of Agricultural Engineering, University of Castilla-La Mancha, Ronda de
Calatrava, s/n, 13003 Ciudad Real (SPAIN)

HIGHLIGHTS

- Application of EKPR increased up to 27% and 7% as compared to natural attenuation and phytoremediation treatments
- 11-21% of the initial atrazine was accumulated in the ryegrass tissues
- Approximately 80% of the atrazine was removed in the first 4 days of treatment

ABSTRACT

Atrazine (ATR) continues being one of the most frequently detected pesticides in natural waters and soils. In this work, an electrokinetic-assisted phytoremediation pot test (EKPR) was performed for the remediation of an atrazine-spiked soil; a low electric voltage gradient (1 V cm^{-1}) with two different electric field operation times (6 and 24 h per day) was used in combination with ryegrass (*Lolium perenne* L.). EKPR increased up to 27% and 7% the overall ATR removal from soil as compared to natural attenuation and phytoremediation treatments, respectively. ATR soil concentration vs time curves were fitted to a pseudofirst-order kinetic equation, obtaining ATR half-life values of 8.2, 7.1 and 5.4 days for the treatments corresponding, respectively, to 0, 6 and 24 h day^{-1} of electric current application. It clearly showed that the ATR removal from soils was enhanced by the electric field. ATR plant accumulation was significantly improved with respect to phytoremediation when the electric current was continuously applied throughout the experiment (24 h day^{-1}); most of the ATR residues were accumulated in the shoot biomass of the ryegrass plants.

Keywords: atrazine, ryegrass, electrokinetic-assisted phytoremediation, removal kinetics

1. INTRODUCTION

The contamination of productive soil and fresh water by pesticides has become a real hazard to the environment and therefore also for living beings (Risco et al., 2016b; Rodrigo et al., 2014). Atrazine, a selective herbicide for the control of broad leaf weeds, is the second most extensively used pesticide in the world with an application of about 70,000-90,000 tons per year in America, Asia and Africa (Cheng et al., 2016; Kumar and Singh, 2016; Singh et al., 2018). Atrazine has a high leaching potential being able to remain in soils and ground waters for long times (Cheng et al., 2016; Kong et al., 2016; Nousiainen et al., 2015; Sass and Colangelo, 2006). Although European Union banned atrazine in 2004 (European Commission Decision 2004/248/EC) and afterwards it was included in the list of priority substances (Directive 2013/39/EU) (Sousa et al., 2018), even today atrazine remains to be one of the omnipresent pollutants in aquatic environments in Europe (Vonberg et al., 2014; Weber et al., 2018).

Based on the success of phytoremediation applied to soils polluted by atrazine (Albright et al., 2013; Merini et al., 2009; Sánchez et al., 2017), we have recently proposed the combination of phytoremediation and electrokinetic remediation to decontaminate atrazine-polluted soils using maize (Sánchez et al., 2019, 2018). Electrokinetic-assisted phytoremediation (EKPR) involves the application of an electric field of low voltage leading to some electrochemical reactions which cause the movement of water, contaminants, free ions, nutrients, etc. through the soil by different mechanisms, i.e. electroosmosis (movement of the soil pore water relative to soil particles), electromigration (transport of dissolved ionic species toward the opposite-charged electrode) and/or electrophoresis (transport of charged particles of colloidal size relative

to the stationary soil pore water) (Cameselle et al., 2013; Yeung and Gu, 2011). This technique may overcome some constraints of the phytoremediation, such as long-time treatment and low availability of pollutants. (Cameselle et al., 2013). Although it has been mainly applied to metal-polluted soils (Aboughalma et al., 2008; Bi et al., 2011; Cang et al., 2012, 2011; Lim et al., 2004; O'Connor et al., 2003; Zhou et al., 2007), Chirakkara et al. (Chirakkara et al., 2015) and Acosta-Santoyo et al. (Acosta-Santoyo et al., 2017) have recently applied EKPR to soils contaminated with both metals and organic compounds (PAHs). Nevertheless, these studies were mainly focused on the germination and survival of plants in the contaminated soil rather than on the specific mechanisms of PAHs degradation. Another important issue that has been scarcely studied is the removal kinetics of organic pollutants from soils by phyto- and/or rhizodegradation (Thoma et al., 2003; Yu and Gu, 2007), although it is possible to find some recent papers about atrazine removal from soils by biodegradation (la Cecilia and Maggi, 2016) and chemical oxidation (Chen et al., 2018). Moreover, to date, there are no kinetic studies about soil remediation by EKPR.

In this paper, the results from an EKPR pot test using ryegrass for the remediation of an atrazine-spiked soil are shown. Two different electric field application times were used in the EKPR experiments with polluted planted pots: 6 and 24 h a day. Another seven control treatments using unpolluted soils, unplanted pots and treatments without electric current (see additional details in Section 2.2) were also used for comparative purposes. Ryegrass was used in this study based on the previous findings in soil phytoremediation (Fang et al., 2001; Lin et al., 2011; Sánchez et al., 2017; Wang et al., 2013) and EKPR (Acosta-Santoyo et al., 2017; O'Connor et al., 2003), its satisfactory growth in clay-textured soils and its relative tolerance to atrazine (Sánchez et al., 2017). This paper is

intended to study: (i) the kinetics of atrazine removal from soils by EKPR using ryegrass; (ii) the effect of the electric current application time on the kinetics and the removal efficiency; (iii) the tolerance of ryegrass to electric current; (iv) the pattern of atrazine accumulation in ryegrass plants and its role in the overall remediation process.

2. MATERIALS AND METHODS

2.1 Soil and plant species

The soil used here was classified as a low plasticity clay soil (CL, according with the Unified Soil Classification System USCS); it came from a quarry located in Mora de Toledo (Toledo, Central Spain; UTM coordinates; X: 431366 Y: 4395232) and had no antecedents of atrazine pollution. It was sampled from a depth between 0.5 and 1.0 m, air-dried at ambient temperature (20-25°C), disaggregated and sieved to 2 mm prior its use. Its main physicochemical properties were: pH of 9.42, a cation exchange capacity (CEC) of 23.42 cmol kg⁻¹, an electrical conductivity (EC) of 0.145 mS cm⁻¹ and a total organic carbon content of 0.60%. Ryegrass (*Lolium perenne* L.) certified seeds were purchased from the Oregon Seed Certification Service (Corvallis, USA). They were pre-germinated in a germination chamber for 3 days at 28 °C using a 0.5 mM calcium sulphate solution. Next, healthy seedlings with uniform size were transplanted into the soil of plastic pots.

2.2 Experimental design

The experiment was carried out in a growth chamber described elsewhere (Sánchez et al., 2017); the conditions used were: 16-h day length, mean day/ night temperatures of 25/15 °C and relative air humidity in the range 60-70%.

FIGURE 1

The EKPR experiment was carried out using a completely randomized design with three replicates (individual pots) per each of the nine different treatments that were conducted. Actually, only two of those treatments corresponded to EKPR tests using planted pots polluted by atrazine (ATR); in these treatments, an electric field of 1 V cm^{-1} was applied 6 and 24 h per day. Planted pots polluted by atrazine but without electric field application were also used as a control (phytoremediation treatment). Additionally, another six control treatments were used for comparative purposes, i.e. pots with plants but no atrazine and pots with atrazine but no plants; both experimental series included the three different electric field application conditions: 0, 6 and 24 h day⁻¹ (Figure 1). An electric field of 1 V cm^{-1} was used in all the pots corresponding to electrical treatments. The atrazine-polluted pots were spiked with an initial ATR concentration of 2 mg per kg of soil (corresponding to $9.273 \mu\text{mol kg}^{-1}$), which corresponds to the typical rates used in agricultural soils ($2\text{-}6 \text{ mg kg}^{-1}$).

One kilogram of air-dried soil was placed in each plastic pot (16 cm bottom diameter, 18 cm depth), obtaining a soil layer of about 6 cm deep. The electric field was applied using a pair of graphite electrodes (7.5 cm in length and 6 mm in diameter) connected to a DC power supply (Delta Elektronika S.V., model SM120-13, The Netherlands). The electrodes were vertically introduced in the soil at both sides of the pots (14 cm between

them and 1 cm from the walls of the pots); the graphite bars were connected to the DC power supply by metal clamps. 300 pre-germinated ryegrass seeds (corresponding to 0.8 g) were sown in each pot.

Fifty two days after ryegrass planting, all the pots were spiked with a 50 mg L⁻¹ atrazine aqueous solution (prepared from a 250 mg L⁻¹ methanol solution of solid atrazine, Sigma-Aldrich, USA) in order to achieve initial soil concentrations of 2 mg kg⁻¹. The volume of the aqueous ATR solution added to each pot (40 mL) corresponded approximately to the water holding capacity of the soil (33%, determined according to the D2980 ASTM method). Similarly, unpolluted pots were irrigated with an equivalent amount of distilled water. The solution was homogeneously scattered on the soil surface and, next, it was allowed to distribute throughout the soil for 24 h before switching on the electric current. Some preliminary tests let to verify that the average ATR soil concentration after 24 h was 1.85±0.12 mg kg⁻¹ (8.55± 0.55 µmol kg⁻¹); this value has been considered as the initial ATR soil concentration in all the figures and calculations. The electric field was applied 6 or 24 hours per day, using periodical reversal of polarity every 2 h in order to avoid the too high or too low pH values typical of electrokinetic treatments (Sánchez et al., 2018). As starting point of the experiment was considered the time in which the electric field was connected (24 h after the spiking of atrazine).

Soil electric current and temperature (measured in the middle of the pot) were recorded daily after the beginning of the experiment. Sampling of soil and plants was conducted using a destructive method; as three samplings were planned, it required to work with 9 initial replicates of each treatment. Thus, three pots of each treatment were sacrificed in days 4th, 10th and 20th after the start of the experiment (Figure 1).

The soil of the each pot was homogenized previously to its characterization. It included the determination of pH, EC and ATR and its metabolites concentrations (see Section 2.3). Ryegrass shoots were harvested by cutting the stem 1 cm above the soil surface. Roots were carefully removed and rinsed with deionized water. Plant samples (shoots and roots) were air-dried at room temperature until constant weight and, finally, milled using a grinder (Retsch Model MM200, Germany). The plant dry biomass was recorded and the plant concentration of atrazine and its metabolites was analysed (see Section 2.3).

2.3 Atrazine analysis

Atrazine and its main metabolites (deethylatrazine, DEA, and deisopropylatrazine, DIA) were determined in soil and plant samples by HPLC, analysing the extracts obtained by a shaking-centrifuging extraction procedure (Amadori et al., 2013). A sample of 2.0 g of soil or plant tissues (previously chopped, crushed and blended) was put in a glass flask; 3 mL of acetonitrile (Sigma-Aldrich, USA) were added and the mixture was shaken for 30 min and centrifuged for 15 min. This procedure was sequentially executed three times and the respective supernatant phases were combined. 0.45 µm nylon syringe filters were used to filtrate the resulting extracts before the HPLC determination. A Shimadzu Prominence UFLC XR (Japan) chromatograph equipped with a C18 reverse-phase column (240 mm x 4 mm) and a diode array detector was used to separate ATR, DEA and DIA. The details of the analytical method used here have been previously reported (Sánchez et al., 2018). ATR, DEA and DIA standard solutions were prepared from the corresponding solid reagents (Sigma-Aldrich, USA).

2.4 Statistical analysis

Statistix 8.1 package (Analytical Software, USA) was used to apply the analysis of variance (ANOVA), corresponding to a randomized complete block design, to all of the data. Mean values of each treatment were compared using the HSD Tukey's test at $p \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1 Variation of soil properties caused by electricity

The electric current was recorded daily in the pots with electric field application throughout the whole experiment. It varied in the ranges 10.0-16.7 and 10-26.67 mA for the electric treatments corresponding to applications of 6 and 24 h a day, respectively (Figure S1). Electric current is closely related with the extent of the electrochemical reactions occurring on the electrode surfaces and the transport of charges through the soil (Risco et al., 2016a). Therefore, since the higher mobilization of charged soil species, electric current was higher when the time of application was increased. Likewise, the current was higher for polluted soils as compared to non-polluted soils in the treatments corresponding to 6 h day⁻¹ of electric field application; this fact may be attributed to the increase of charged species in soils due to the addition of atrazine and its subsequent partial ionization ($pK_a = 1.68-1.71$). For the 24 h day⁻¹ treatment, significant differences between polluted and non-polluted treatments were not found. The electrical conductivity (EC) of the soils is also related with the increase of the

charged species in the soil due to, on one hand, the electrochemical reactions taking place in the electrodes (Cang et al., 2011; Virkutyte et al., 2002) and, on the other hand, the addition of atrazine. So, soil EC (measured after the experiment) was significantly increased for all treatments with electric field application as compared with the EC of the initial soil ($145.7 \mu\text{S cm}^{-1}$) (Table 1). Soil EC values were higher for the treatments with continuous electricity application (as compared to 6 h day^{-1} treatments) and with atrazine (as compared to unpolluted soils). Likewise, the presence of ryegrass led to lower values of soil EC as compared to unplanted pots; it may be attributed to the absorption of ionic species by plant roots (Chirakkara et al., 2015).

The temperature of the soil section located between the electrodes was also recorded daily in the pots with electric field application. The soil temperature values from all pots were in the range $18.5\text{-}23.1^{\circ}\text{C}$ (data not shown), with no significant differences between treatments; it means that there was not a significant influence of the electric field application. The slight variations detected in soil temperature in different days were mainly due to the changes in the temperature of the growth chamber.

TABLE 1

The values of the soil pH after the experiment (day 20th) for the different treatments with electric field application are shown in Table 1. All the values were in the range $8.43\text{-}9.02$, with no significant differences between treatments (critical value of HSD Tuckey's test = 0.576 , $p \leq 0.05$). Therefore, it seems that periodic electric polarity changes prevented the extreme soil pH values which are characteristic of the electrokinetic remediation processes (Reddy and Cameselle, 2009); the potential buffer

effect due to the presence of calcite in the used soil (data not shown) could have also contributed to avoid pH changes in some extent. Thus, in good agreement with our previous findings, the lowest value of the soil pH changed the initial soil pH only about one unit (9.42) (Sánchez et al., 2019). The observed slight soil acidification could be due to the higher mobility of H^+ ions generated in the anode reaction ($2 H_2O \rightarrow O_2 + 4 H^+ + 4 e^-$) as compared to hydroxyl ions generated in the cathode ($2 e^- + 2 H_2O \rightarrow 2 OH^- + H_2$) (O'Connor et al., 2003).

3.2 Atrazine removal from soils: kinetics

FIGURE 2

TABLE 2

In Figure 2 are plotted the soil concentration of atrazine (ATR) and its metabolites vs time for the different treatments. Firstly, it can be seen that, independently of the total amount of ATR eliminated, most of it (67.3-86% of the initial ATR) was removed in the first 4 days of the experiment for all the treatments, ; later, ATR soil concentration decreased in a much less pronounced way, especially for planted pots.

Natural attenuation (treatment with no plants and electric current) led to a final parent atrazine removal (without considering the ATR metabolites remaining in the soil) of 67.5% (after 20 days of treatment). This removal efficiency was increased to 80.0-83.0% for treatments with electrokinetic remediation (no plants and with electric current), to 87.0% for phytoremediation (plants without electric current) and to 89.5-

94.4 % for electrokinetic-assisted phytoremediation; the differences between treatments were statistically significant (Table 2). It clearly shows the improvement in atrazine degradation by the combination of electrokinetic and phytoremediation technologies using ryegrass, in a same way that we had previously reported for the EKPR using maize (Sánchez et al., 2019, 2018). In fact, the highest value for atrazine removal was obtained for the EKPR treatment with continuous application of electric current (24 h day⁻¹); it reached a value of 94.4%, taking only into account the removal of the parent atrazine, and 66.2% if all the ATR chemical species remaining in the soil (atrazine and its metabolites) are considered (Table 2).

Together to the removal of atrazine from soils, significant soil concentrations of ATR metabolites (DEA and DIA), in similar or higher values than those belonging to parent ATR, were found from the first sampling (day 4th, Figure 2). It means that microbial degradation of atrazine to deethylatrazine and deisopropylatrazine (and perhaps hydroxyatrazine) was an important mechanism of ATR removal from soils in our experiments, both in unplanted and planted treatments. Such pathway of ATR elimination has been previously reported by other researchers (Krutz et al., 2008; Cecilia and Maggi, 2017; Lim et al., 2004; Mudhoo and Garg, 2011) and it was also suggested by us for the electrokinetic-assisted phytoremediation of atrazine-polluted soils using maize (Sánchez et al., 2019, 2018). Since DEA and DIA are less toxic than the parent atrazine, this degradation pathway can be considered of a mechanism of soil detoxification (Fan and Song, 2014).

On the other hand, as it can be seen in Table 2, the total amount of ATR residues (atrazine and its metabolites) accumulated in ryegrass tissues involved a significant part

of the total ATR removed, i.e. 10.9-20.8% of the initial ATR (equivalent to 17-34% of the ATR removed), showing the valuable role of ryegrass in the atrazine removal process. It will be described in more detail in the next section. ATR mineralization inside the plants (phytodegradation) and/or in the rhizosphere (rhizodegradation), although not registered in our experiment, should also be taken into account to appreciate adequately the overall contribution of plants to soil decontamination.

Atrazine removal from soils by EKPR is a very complex process in which a number of physical, chemical and biological phenomena are involved; moreover, the complex nature of the interactions soil-plant-microorganisms should also be considered. Consequently, the development of a detailed kinetic model is a difficult task that clearly exceeds the objectives of this research. Nevertheless, data of soil atrazine concentration vs time from Figure 2 were fitted to a simple pseudofirst-order kinetic equation in order to obtain the removal rate constant (K_{obs}) and the ATR half-life ($t_{1/2}$) values corresponding with the different treatments conducted in this work. This type of models has been applied to simulate the fate of atrazine (Chen et al., 2018; Prado et al., 2014) and other pesticides in the environment (Beulke and Brown, 2001). The results of the data fitting are shown in Table 3.

TABLE 3

In spite of the empirical character of the fitting and the relative low values of some correlation coefficients (0.57-0.78), some interesting remarks may be pointed out from the results in Table 3. Firstly, it can be seen that the values of K_{obs} corresponding to the treatments with ryegrass were much higher than those of unplanted ones (between 52

and 83%). Moreover, for planted pots, the value of K_{obs} corresponding to the phytoremediation treatment (0.084 days^{-1}) was significantly increased with the electric field application, i.e. 0.128 days^{-1} for continuous application of electric current. Those values led to ATR half-life values in the range 9.1-15.1 days for unplanted treatments, while this range was 5.4-8.3 for treatments with plants; the lowest value corresponded with the continuous electric field application (24 h day^{-1}). ATR half-life values from 4 to 385 days have been reported in natural soils depending on specific field conditions, while laboratory biodecomposition tests have led to half-life values of 3-6 days (la Cecilia and Maggi, 2016). So, the data obtained here seem to agree with those values.

The presence of ATR metabolites in the soil followed a different trend depending on the treatment applied (Figure 2). For planted pots and those without plants and electric current, ATR metabolites concentrations were very similar to ATR concentrations for the day 4th and, after that, they were progressively increased in a similar extent than the atrazine concentrations decreased. It seems that after 4 days of treatment, atrazine uptake by plants began to slow down and the further decrease of soil atrazine was mainly due to microbial degradation. This finding agrees with the progressive decrease of atrazine plant accumulation observed as the experiment progressed (Table 2). It could be related to the decrease in atrazine availability as the experiment progresses. However, for unplanted pots with electric current application, soil ATR metabolites concentrations corresponding to the day 4th were much higher than ATR ones; they decreased from day 4 to 10 and, finally, they were increased in a similar extent than soil atrazine decreased. It means that the mineralization rate of ATR metabolites was higher than atrazine degradation from day 4 to 10 and, next, it was slowed down; the reason of it could be the progressive plant stress caused by atrazine absorption and the electric

current application (see next Section). Both processes, i.e. atrazine degradation and ATR metabolites mineralization, seemed to be accelerated by the electric current application.

3.3 Plant biomass and atrazine accumulation

FIGURE 3

Figure 3 shows the values of root, shoot and total plant biomass (g dry matter pot⁻¹) corresponding to days 4th, 10th and 20th after the beginning of the experiment.

In general, one can expect that plant biomass was increased over time as a consequence of a healthy growth. On the contrary, a decay in biomass should be indicative of plant stress. It can be seen in Figure 3a that plants grown in an unpolluted soil and without electric current application increased their root and shoot biomass, in approximately 6 and 30%, respectively, throughout the experimentation time. However, the application of electric current to the unpolluted soils led to a decrease of the root biomass from the first plant sampling (in day 4th). For the continuous electric field application, the decrease of root biomass was kept until the end of the experiment leading to a significant decrease of 32% in the total ryegrass biomass; however, shoot biomass was not significantly affected (Figure 3a). When the electric field was applied only 6 h a day, root biomass was affected by the electric current in the first days, but after that, it increased slightly over time. Moreover, shoot biomass was slightly increased with respect to the control. On the overall, the application of electric current 6 h a day led to a non-statistically significant biomass decrease of 9.1% (Figure 3a).

Otherwise, the spiking of soils with 2 mg kg⁻¹ of atrazine only affected significantly to plant root biomass, which decreased in approximately 38% for pots without electric current after 20 days of experiment (Figure 3b). In a same way than in unpolluted pots, the application of the electric field to the polluted ones led to a decrease in root biomass, higher for the 24 h day⁻¹ application. Shoot biomass was less affected by atrazine pollution and electric field application than root biomass, but it was continuously decreased throughout the experiment, indicating plant stress. As a result, total plant biomass decreased continuously over time due to the spiking of pots with atrazine; on the whole, total ryegrass biomass was decreased in 34, 35 and 22% with respect to unpolluted pots for 0, 6 and 24 h day⁻¹ of electric current application, respectively.

Therefore, we can conclude that both aspects, atrazine pollution and electric field application, had negative and crossed effects on ryegrass growth, those being more pronounced for roots. Atrazine has been previously reported to cause some visual symptoms of toxicity, such as chlorosis and stunting, to ryegrass, but it did not lead to the death of plants (Fang et al., 2001; Sánchez et al., 2017). Zhou et al. (Zhou et al., 2007) reported that shoot and root biomass of ryegrass decreased around 15% and 50% when a DC electric field of 1 V cm⁻¹ was applied during 7 days, as compared to pots without electricity. However, a more recent work by Acosta-Santoyo et al. (Acosta-Santoyo et al., 2017) concluded that the application of a low electric field (0.2 V cm⁻¹) improved the ryegrass germination in unpolluted soils, while it compensated the harmful effects of metals and PAHs in polluted soils. Lastly, it must be mentioned that Cang et al. (Cang et al., 2011) found a more relevant response to electric current for the

root biomass of Indian mustard as compared with the aboveground biomass; it agrees with our findings for ryegrass.

FIGURE 4

Figure 4 shows the concentration and accumulation of atrazine and its metabolites in plant tissues in days 4th, 10th and 20th from the beginning of the experiment. Firstly, it must be pointed out that atrazine and its metabolites were mainly accumulated in the aerial part of ryegrass for all the treatments and sampling times; in fact, the values of the shoot:root concentration ratio were in the range 11.0-68.7. The application of an electric field does not seem to affect this atrazine accumulation pattern, which has been previously reported to be specific of each plant species (Henderson et al., 2007; Murphy and Coats, 2011; Sánchez et al., 2017).

The highest values for atrazine plant accumulation were reached for the first sampling day (4th) and they were further decreased throughout the experiment (Figure 4). However, those decreasing patterns were different depending on the type of treatment. When no electric current was applied, the amount of atrazine in the plants decreased in an approximately linear trend for days 4th, 10th and 20th of treatment; for this treatment, atrazine plant concentration decreased between days 4 and 10 but, then, it was increased by day 20. The pattern for the application of the electric field 6 h a day was some different, since atrazine accumulation decreased sharply between days 10 and 20; however, ATR plant concentration exhibited a continuous decreasing pattern throughout the experiment. Finally, when the electric field was applied continuously, atrazine plant accumulation decreased markedly between days 4 and 10 and, later, it

remained approximately constant by day 20; in this case, ATR plant concentration also decreased between days 4 and 10 and, then, it increased sharply until the end of the experiment. Atrazine has been previously reported to be readily taken up by plants (Wenger et al., 2005), even one day after its application (Albright et al., 2013). Plant uptake of organic compounds has shown to be favoured when the logarithm of the octanol-water partition coefficient ($\log k_{ow}$) is in the range 0.5-3.0. Atrazine, with a $\log k_{ow}$ of 2.68, could be partially taken up by roots and transferred through cell membranes to reach plant shoots (Briggs et al., 1982; Cejudo-Espinosa et al., 2009; Romeh, 2015).

In contrast to the electrokinetic-assisted phytoremediation of soils polluted by metals, in which the success of electric current application may be assessed in terms of a higher metal accumulation in plant tissues, EKPR of organic pollutants may or not lead to higher plant concentrations than those of phytoremediation. This fact may be explained because organic pollutants can not only be taken up and accumulated in plant tissues but also degraded within the plants; in fact, N-dealkylation (yielding DEA and DIA metabolites) has been shown as the most important detoxification mechanism for triazine herbicides in several C_3 species, such as annual ryegrass (Burken and Schnoor, 1997). In our experiment, the presence of ATR metabolites in the ryegrass tissues for all the treatments and samplings seems to demonstrate that atrazine degradation took place inside the plants in some extent; it is also supported by previous findings regarding atrazine accumulation in plants (Fan and Song, 2014; Lin et al., 2008; Murphy and Coats, 2011). Nevertheless, the experimental methodology used here does not let to discard the uptake of ATR metabolites from soil.

In any case, concentration and total accumulation of atrazine in plants will be dependent on both plant uptake and phytodegradation rates together with plant biomass. The highest values for atrazine plant accumulation and concentration were found for the treatment corresponding to 24 h day⁻¹ of electric field application; therefore, it shows the success of EKPR to increase the mobility and availability of atrazine in soils. There are no reasons to suppose that electric current did not also improve atrazine mobility and plant uptake in the treatment with only 6 h day⁻¹ of electric current application. The increasing ATR concentration and accumulation values throughout the experiment seems to support that hypothesis (Figure 4). The better balance between ATR plant uptake and plant degradation in the treatment corresponding to 6 h day⁻¹ would explain the lower values for ATR concentration and accumulation as compared to those of the 24 h day⁻¹ treatment. In this latter case, the rate of atrazine plant uptake, caused by a higher availability of atrazine, would exceed the degradation rate, leading to increasing ATR plant concentrations between days 10 and 20. The increasing of plant stress as the experiment progresses would be also responsible for the slowing down of plant degradation observed for the 24 h day⁻¹ treatment. The significant decrease found in plant biomass for this treatment (Figure 3b) between days 10 and 20 contributed to keep approximately constant the plant ATR accumulation in spite of the rise in the plant ATR concentration (Figure 4). Lastly, the increase of ATR metabolites between days 10 and 20 for the 6 h day⁻¹ treatment would give additional evidence on the phytodegradation rate improvement caused by the electric field application during few hours a day.

4. CONCLUSIONS

From this work the following conclusions were drawn:

- The application of an electric field of 1 V cm^{-1} with periodic polarity reversal every 2 h led to a decrease of the soil pH less than one unit.
- Application of EKPR increased the removal of atrazine from soils up to 27% and 7% as compared to natural attenuation and phytoremediation treatments, respectively. Approximately 80% of the initial atrazine was removed from soils in the first 4 days of treatment.
- Fitting of experimental data of atrazine soil concentration vs time to a pseudofirst-order kinetic equation let to obtain the values of the ATR removal rate constants (K_{obs}) and the ATR half-life ($t_{1/2}$) for the EKPR tests.
- The application of electric current to pots with ryegrass led to decrease of plant biomass by 9.1% and 32% for application times of 6 h day^{-1} and 24 h day^{-1} , respectively, as compared to pots without electric current application.
- Atrazine residues were mainly accumulated in the aerial part of ryegrass; it accounted for 10.9-20.8% of the initial ATR (equivalent to 17-34% of the ATR removed), showing the important role of ryegrass in the overall atrazine removal.

Finally, it must be pointed out that the results reported here, obtained using spiked soils, should be carefully extrapolated to real conditions in which the aging of the pollutants could have a determinant role in the effectiveness of the proposed treatment.

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Table 1. Soil pH and electrical conductivity ($\mu\text{S cm}^{-1}$) at the end of the EKPR experiments. Values are expressed as mean $\pm$ SD of three replicates. Different letters indicate significant differences between treatments

Treatments			Soil properties	
Initial dose ATR (mg kg ⁻¹ soil)	Plant	Electric field (h day ⁻¹)	pH	Electrical conductivity (μS cm ⁻¹)
0	Ryegrass	6	8.88±0.40	181.50±5.36a
		24	9.02±0.16	198.73±2.64b
2	Ryegrass	6	9.03±0.00	207.67±2.52b
		24	8.97±0.19	226.47±4.84c
	Control	6	8.76±0.10	264.43±3.19d
		24	8.43±0.06	270.00±6.00d
Significance level†			NS	*
Critical value for comparison (HSD Tuckey's test)			0.576	11.57

† * denote significant differences at $p \leq 0.05$

NS: no significant differences.

Table 2. Distribution of atrazine and its metabolites (% of the initial soil ATR) at the end of the EKPR experiments.

Values are given as the mean $\pm$ SD (n=3)

Time (days)	Plant	Electric field (h/day)	% of the initial ATR					
			Soil			Plant accumulation		
			ATR	Metabolites	Total ATR	ATR	Metabolites	Total ATR
4	Ryegrass	0	19.40±0.73	19.61±0.53	39.01±0.33	22.52±1.20	3.83±0.34	26.35±1.22
		6	23.18±0.12	17.26±0.79	40.44±0.75	16.85±0.53	2.60±0.49	19.45±0.64
		24	21.07±0.04	21.51±0.30	42.57±0.28	30.86±1.33	3.82±0.18	34.68±1.25
	Control	0	39.93±0.58	43.01±0.06	82.94±0.50	-	-	-
		6	25.64±0.11	49.91±3.74	75.55±3.36	-	-	-
		24	32.70±1.05	42.74±0.19	75.44±0.87	-	-	-
10	Ryegrass	0	13.56±0.68	25.38±0.04	38.94±0.37	20.73±0.94	2.31±0.14	23.03±1.01
		6	17.43±1.21	20.25±0.71	37.68±0.46	14.31±0.80	2.11±0.31	16.42±0.90
		24	8.32±0.76	29.90±0.34	38.22±0.82	18.04±2.37	2.52±0.12	20.56±0.74
	Control	0	34.97±0.61	47.71±2.87	82.68±2.19	-	-	-
		6	23.50±0.30	37.78±0.58	61.28±0.71	-	-	-
		24	26.95±0.60	30.02±0.69	56.97±1.12	-	-	-
20	Ryegrass	0	13.02±1.17	25.81±0.09	38.84±1.72	18.49±2.73	2.27±0.39	20.76±2.51
		6	10.47±0.20	26.92±0.74	37.40±0.50	7.74±1.00	3.16±0.67	10.90±1.54
		24	6.02±0.08	30.62±0.60	36.65±0.57	17.31±2.53	1.97±0.18	19.28±2.69
	Control	0	32.55±0.15	49.86±1.82	82.41±1.80	-	-	-
		6	20.04±0.05	39.74±1.73	59.78±1.56	-	-	-
		24	16.99±0.06	39.75±0.52	56.74±0.43	-	-	-
Significance level†			***	***	***	*	**	NS
Critical value for comparison (HSD Tuckey's test)			1.72	4.01	3.98	7.05	1.02	7.96

† *, **, and *** denote significant differences at $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$, respectively. NS: no significant differences.

Table 3. Kinetics of atrazine removal from soils: pseudo-first order kinetic parameters. (K_{obs} : removal rate constant, day⁻¹; $t_{1/2}$: atrazine half-life, day)

Initial dose ATR (mg kg⁻¹ soil)	Electric current (h day⁻¹)	Plant	K_{obs} (day⁻¹)	$t_{1/2}$ (day)
2	0	Ryegrass	0.084	8.25
		Control	0.046	15.07
	6	Ryegrass	0.097	7.14
		Control	0.064	10.83
	24	Ryegrass	0.128	5.41
		Control	0.076	9.12

FIGURE CAPTIONS

Figure 1. Experimental design and pictures of the applied treatments.

Figure 2. Soil atrazine concentration (ATR, ATR metabolites and total ATR) over time for the EKPR experiments (planted/unplanted soils; electric field application: 0, 6 and 24 h day⁻¹). Vertical lines represent critical values for comparison according HSD Tuckey's test ($p \leq 0.05$): (a) atrazine; (b) ATR metabolites; (c) total ATR.

Figure 3. Root, shoot and total biomass (g pot⁻¹) reached by ryegrass over time for the EKPR experiments (electric field application: 0, 6 and 24 h day⁻¹; planted non-polluted/polluted soils). Vertical lines represent critical value for comparison according HSD Tuckey's test ($p \leq 0.05$): (a) root; (b) shoot; (c) total plant.

Figure 4. Atrazine residues (ATR and its metabolites) accumulation (bars) and concentration (lines) in ryegrass over time for the EKPR experiments (planted soils; electric field application: 0, 6 and 24 h day⁻¹). Vertical lines represent critical value for comparison according HSD Tuckey's test ($p \leq 0.05$): (a) ATR accumulation; (b) ATR metabolites accumulation; (c) ATR concentration; (d) ATR metabolites concentration.

FIGURE 1

FIGURE 2

FIGURE 3

FIGURE 4