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Electro-bioremediation of a mixture of structurally different contaminants of emerging concern: Uncovering electrokinetic contribution

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ABSTRACT

This study analyses the electrokinetic (EK) contribution to the removal from a clay soil of a mixture of 10 different contaminants of emerging concern (CECs; 17β-estradiol, E2; sulfamethoxazole, SMX; bisphenol A, BPA; ibuprofen, IBU; 17α-ethinylestradiol, EE2; oxybenzone, OXY; diclofenac, DCF; triclosan, TCS; caffeine, CAF; carbamazepine, CBZ). After 4 days, the CECs natural attenuation was between 0% (CBZ) and 90% (E2) yet increasing with the application of EK (20 mA, 12 h ON/OFF) to 14% (CBZ) and 100% (E2). When EK was applied, the CECs more recalcitrant to biodegradation (*i.e.* ≤13% biotic decay) mostly underwent electro-chemical induced degradation (OXY, DCF, TCS, CAF, CBZ). Daily irrigation enhanced the rates of the electro-oxidation -osmosis and -migration, increasing the CECs decay. After 8 days of EK treatment, the CECs decay increased, surpassing the decay *lag* phase of some compounds (OXY, TCS, and CBZ). Yet after 16 days, most CECs showed similar removals with and without EK, with EK only acting positively on SMX, OXY, TCS and CBZ (*ca*. +10%). Our results support that EK application can improve the removal of CECs from soil, however, under the conditions tested, 16-day treatment lead to pH alterations that decreased the bioremediation efficiency and inhibited electro-degradation near the cathode.

Keywords: electrokinetic remediation; microbiota; abiotic removal; clay soil; pharmaceuticals and personal care products

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1. INTRODUCTION

Soil, a non-renewable resource, is a base for life and for supporting livelihoods. Soil protection is critical to ensure food provision, store and supply more clean water, maintain biodiversity, sequester carbon and increase resilience to climate change. To reach this goal requires the global implementation of sustainable soil management practices [1]. Nowadays, concerns about soil contamination are growing worldwide as it can severely degrade the major ecosystem services provided by soil and consequently affecting human and environmental health.

The use of effluent, reclaimed wastewater (RWW), for irrigation increases the potential risks associated with the prevalence and environmental dissemination of diverse contaminants of emerging concern (CECs), including pharmaceutical and personal care products [2]. The presence of these contaminants in RWW is not complied in its usage requirements [3–5]. Thus, when RWW containing CECs is used, those contaminants can accumulate in the soil, be transferred to the food chain and leach to ground and surface waters, carrying unexpected risks to both the environment and human health [6–8]. One of the biggest problems, nowadays, associated with the presence of CECs in the environment, is the fact that some of these contaminants have the potential to promote bacterial resistance in the environment [9–11]. Several technologies are currently being developed for wastewater treatment, especially aiming to ensure the removal of target CECs [12]. To date their removal from soils remains overlooked, regardless that CECs are found in soil worldwide, emphasizing the urgency of developing novel effective and economically sustainable remediation treatments for CECs removal *on-site*.

Bioremediation is one of the most cost-effective remediation methods for contaminated soils [13]. Bioremediation relies on the functioning of the soil microbiota hence largely influenced by the environmental conditions that might be favorable or not for specific biochemical processes and interactions between microorganisms, contaminants and nutrients to occur [14]. The efficacy of the bioremediation process varies accordingly to the type of soil, available medium conditions and contamination present at the targeted site. For example, impermeable soil exchanges very little amount of oxygen and nutrients, limiting the bioavailability of the contaminants, consequently the remediation rate is very slow to ensure timely mitigation of ecosystem associated-risks [14]. Some

limitations of bioremediation can be overcome, namely in non-permeable soils, by coupling it with the electrokinetic (EK) process.

The EK process has been successfully used for the treatment of contaminated soils with organics, heavy metals and other inorganic contaminants [14–17]. This remediation technique involves the application of a low level direct current across electrodes inserted in the soil [18]. Through the generated electric field, electroosmosis, electromigration and electrophoresis occurs, as well as the electrolysis of water that generates H^+ and OH^-. Thus, when applied at mild conditions, EK has the potential to increase biodegradation efficiencies, mainly through the mobilization of pollutants and, also, in the case of organic contaminants, by promoting their degradation *via* induced electro-chemical degradation (reduction and oxidation) [14,19,20]. Simultaneously, EK usage might stimulate both the mobility and the activity of bacteria in soil [14].

The application of the EK process coupled to bioremediation for the removal of CECs from soil is still in an early stage of development [14,19–21]. Despite some promising results, little is still known about the degradation pathways and mechanisms involved in the EK enhanced bioremediation of different CECs. Finally, the application of EK should influence the distribution (*i.e.* mobilization) of the contaminants and of the microbiota along the soil column. Understanding these important phenomena becomes essential to seek the development of an effective synergy between EK and the soil microbiota that improves the overall remediation efficacy. In a previous study we found that the application of EK in a 12 h ON/OFF mode enhanced the decay of a mixture of 4 CECs contaminating a clay soil, including some very recalcitrant [19], possibly due to increased CECs mobilization since their transfer from the soil into the interstitial fluid (*i.e.* solubilization and desorption) increases in the void current periods [22]. Using the team established know-how, this study aims to better understand the effective contribution of EK in the degradation of a complex mixture of 10 CECs, spiked in a clay soil. The selected CECs (ESI 1) are usually found in RWW, surface water and soils, namely 17β-estradiol (E2; naturally occurring estrogen), sulfamethoxazole (SMX; bacteriostatic antibiotic), bisphenol A (BPA; plasticizer), ibuprofen (IBU; an analgesic, nonsteroidal anti-inflammatory drug), 17α-ethinylestradiol (EE2; semisynthetic estrogen), oxybenzone (OXY; sunscreen agent), diclofenac (DCF; an analgesic, nonsteroidal anti-inflammatory drug, triclosan (TCS; an antimicrobial disinfectant), caffeine (CAF; a central nervous system stimulant) and carbamazepine (CBZ; anticonvulsant) [23–

25]. Specifically, we have assessed the *short-term* (4 days) effect of EK application on both mobilization and degradation of the CECs mixture, under biotic or abiotic conditions, with or without irrigation. A comprehensive analysis of the soil physicochemical properties (viz. soil temperature, moisture, pH and conductivity) was also undertaken. Finally, we measured the effects of *longer* EK application (till day 16) on the soil properties, including the persistence of the CECs, after eight and sixteen days of treatment.

2. MATERIALS AND METHODS

2.1. Chemicals and solvents

All chemicals, including standards and solvents (all analytical grade) were purchased from Sigma–Aldrich (Steinheim, Germany), except triclosan that was purchased from Labesfal Farma (Tondela, Portugal). The water was deionized and purified with a Milli-Q plus system from Millipore (Bedford, MA, USA).

2.2. Soil sampling

The soil (0–15 cm depth) was collected in an organic tomato farm in São Nicolau, Santarém, Portugal (39°12'42.6"N, 8°42'41.5"W) in October 2017. Prior to use, the soil was sieved (No. 10 IS Sieve, 2.0 mm) to remove the coarse fractions, and its physicochemical characterization was undertaken. It presents a clay texture (61% clay, 29% silt and 10% sand) and a high content of mineral and organic colloids (see full details in ESI 2).

2.2.1. Soil sterilization

Sterilization of soil samples (coded as Abiotic) was performed in a flow chamber under direct UV light for 2 h, followed by 6 times 1 h cycle at 121 ºC in an autoclave. The sterility of the soil was confirmed prior to the experiments by inoculating solid media with soil extracts (*see below*) microbial development was not observed even after 7 days.

2.3. Electrokinetic microcosm set-up

In a previous study we have resorted to graphite electrodes that were unstable at currents above 10 mA [19]. In the present study and with the purpose to increase the applied current, we resorted to mixed metal oxide (MMO) electrodes that have shown potential to induce CECs degradation [26,27].

The experimental microcosm (Figure 1a) consisted of a rectangular acrylic container (100 x 50 x 70 mm), in which two of the selected electrodes (MMO mesh, Force ®; 60 x 20 x 0.5 mm) were inserted laterally, 5 mm from the edge and connected to a power supply for direct current generation (Hewlett Packard E3612A, Palo Alto, USA). For microcosms assembly, a total of 345 g of soil (60 mm height) was poured in 3 consecutive layers: the bottom two layers were watered (5:1, m:V), and the topsoil layer was irrigated with water (5:1, m:V) spiked with the mixture of 10 CECs to account ~4 mg of each CEC *per* kg d.w. of soil (aiming to simulate soil contamination due to irrigation with contaminated RWW). The current used in the EK processes was set to 20 mA (0.27 mA/cm^2) with an ON/OFF switch of 12 h, *i.e.* a continuous application of identical periods of current and void.

Two experimental rounds were carried out (six experiments each; protected from direct UV light; duplicate experiments; ESI 3):

- Round 1, *short-term* experiments - 4 days aiming to assess CECs decay using sterilized soil (Abiotic) versus non-sterilized soil (Biotic) inside a flow chamber (sterile conditions; 23.5 ± 0.5 ºC): the biotic and the abiotic controls (i.e. without EK), plus two EK-Abiotic and two EK-Biotic with or without daily irrigation (W; by dropping water on the topsoil layer, 5:1 m:V).
- Round 2, *longer* experiments - 4, 8 and 16 days aiming to assess CECs decay along time at room conditions (non-sterile conditions; 19.0 ± 0.5 ºC): three biotic controls (i.e. without EK) and three EK-Biotic tests, all under the same irrigation regime (irrigated every 4 days with deionized water by dropping the deionized water on the topsoil layer; 5:1 m:V).

In each EK experiment the current intensity, the voltage between the electrodes, and the soil temperature was daily monitored. At the end of the experiments, the soil was carefully removed and segmented into 6 sections across distinct planes (Figure 1b): transverse (layers 1, 2 and 3; to assess CECs leaching potential) and tangential (anode section - A; cathode section - C), which were immediately processed and analyzed (see section 2.4). In parallel, the time zero control was also analyzed and processed immediately after spiking the soil with the CECs mixture.

2.4. Analytical methodologies

2.4.1.Physicochemical parameters

The soil temperature was measured with a digital thermometer (middle point of the two electrodes, 1 cm depth). The soil water content was determined as the weight loss after 24 h at 105 ºC till constant

weight. Conductivity and pH were evaluated using a soil deionized water ratio of 1:2.5 (w:V), stirred for 1 h, then left aside to settle for another hour prior measurement, with the pH meter Metrohm-Solitrode with Pt1000 and the Horiba-LAQUAtwin conductivity meter. Each sample was measured in duplicate.

2.4.2.Organic contaminants extraction

The concentration of the organic compounds was determined using an adapted QuEChERS method described in Guedes *et al.* [19]: 2.5 g of soil collected right after the end of the experiment was mixed with 1.5 mL of deionized water (vortex 30 sec); then 2.5 mL of acetonitrile was added (vortex 1 minute); and then 1 g of $MgSO_4$ (mixed vigorously manually, followed by vortex 30 sec). The supernatant (organic phase) was recovered after centrifugation (2800 x *g*, 5 min; room temperature), filtered through a PTFE syringe filter 0.45 µm (previously passed through acetonitrile), transferred to a vial and conserved at -20 ºC. Each sample was extracted in triplicate. Extraction method recoveries were between 85-105% (ESI 4).

2.4.3.CECs quantification using HPLC-DAD-FLD analysis

Analyses were performed using high-performance liquid chromatography (HPLC) with diode array detector (DAD) and fluorescence detector (FLD). HPLC analysis was performed on a LC System equipped with a Quaternary Pump (G7111B) and a vial sampler (G7129A) (Infinity II, 1260 Series, Agilent Technologies, USA), coupled to a diode array detector (G1315B) and a fluorescence detector (G1321A) (Agilent 1100 Series). The UV wavelength was set to scan from 200 to 500 nm and the fluorescence was measured with excitation at 220 nm and emission at 290 nm. The system was operated using the LC OpenLab software (version 2.15.26).

Analytes separation was performed using a Poroshell 120 EC-C18 2.7 µm column with 4.6 x 100 mm from Agilent (California, USA), and an Onyx SecurityGuard C18 cartridges, with 5 x 4.6 mm, from Phenomenex (Torrance, USA). The oven was set to 36 ºC. The HPLC runs were performed at a constant flow of 1.5 mL/min, in gradient mode. The eluents used were [Water]/[Acetonitrile]/[50% Formic acid in water] as follows: eluent A: 94.5/5/0.5; eluent B: 5/94.5/0.5. All eluents were filtered through Nylon 66 membranes (pore size of 0.45 µm; Bellefonte, PA, USA). The gradient run was set to 1 min 5% B, after 95% B until 9 min, then 97% B until 10 min, where it was held constant for 2 min and then to 5% B until 13 min. Post run equilibrium was carried out for 2 min.

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Preceding analysis, each soil extract was mixed with eluent A (2:1) in a vial with insert and analyzed. The target CECs were quantitatively analyzed at: 282 nm for CAF, SMX, CBZ, DCF, OXY and TCS concentration; and 220-290 nm of excitation emission for BPA, E2, EE2 and IBU. Data analyses were processed using the LC OpenLab software. Repeatability presented a coefficient of variation between 10 and 28% whereas intermediate precision was between -1 to +12%. The matrix effect caused deviations of HPLC quantification which were estimated to be between -3 to +15% (data not show). The limits of detection and quantification (LD and LQ, respectively) can be found in ESI 4.

2.4.4.Degradation kinetics

The CECs biotic decays along time, round 2 experiments, were adjusted to a pseudo first-order model, as follows equation 1:

$$\ln(C/C_0) = -kt \quad (1)$$

where C and C_0 represent the concentration (µg/kg d.w.) of the target CEC at time t and zero, respectively, k is the degradation rate constant (h^{-1}). Then, half-lives ($t_{½}$) were calculated according to equation 2:

$$t_{1/2} = \ln(2)/k \quad (2)$$

For the CECs that we observed the occurrence of a *lag* phase (microbiota acclimation) followed by a biodegradation phase, the half-life times ($t_{½}$) were obtained by adding the *lag* phase to the half-life values, calculated from the first order kinetic constant (k) [28,29], and then the data were fitted to a second degree polynomial model previously used for surfactants biodegradation in waters [29] according to equation 3:

$$-\frac{\partial C}{\partial t} = K_2 \cdot C^2 + K_1 \cdot C + K_0 \quad (3)$$

where C is the compound concentration at time *t*, K_2 is the coefficient of C^2, K_1 is the coefficient of *C*, and K_0 is an independent term that represents the concentration of non-biodegradable substrate in the medium.

2.5. Statistical analysis

Significant differences among the samples (95% confidence interval, $p<0.05$) were evaluated through one-Way ANOVA Tukey's multiple comparisons test, using GraphPad Prism software (version 8). The multiple comparison analysis was designed to extract the degree of dissimilarity among the experimental observables after the treatment (*viz.* physical parameters of the soil and the quantities of each CEC) *per* soil segment (soil sections, Figure 1b) relative to the time zero control. We compared different soil sections of the same experiment but also the equivalent soil sections of different experiments. Samples with CEC below the limit of quantification of the method (yet close to the limit of detection) were designated as not detected (n.d.) and accounted as "0" for the statistical analyses, except for samples devoid of a quantifiable duplicate which were disregarded.

3. RESULTS AND DISCUSSION

3.1. *Round 1: Short-term* effects of EK application

3.1.1. Initial characterization of the EK system (pH, conductivity, temperature, water content, voltage)

When no EK was applied regardless of the presence or not of the soil microbiota, in general stable pH values were observed after 4 days (Table 1), with a mean value of 8.04 ± 0.10 ($p>0.05$). As expected, when EK was applied, the pH was different across both length and depth of the soil layer due to the water electrolysis that produces H^+ in the anode and OH^- in the cathode. These pH changes were more pronounced in the cathode side, where a trend for pH increase with depth was observed (Table 1). For example, in the EK-Abiotic test, the pH was 8.84 in the topsoil layer near the cathode (section C1) and 9.85 and 10.44 in the sections below (C2 and C3), with statistically significant differences ($p<0.05$). The biggest pH difference observed between anode and cathode soil sections was 3.5 units registered in EK-Abiotic in the bottom soil layer, that ranged from 6.94 near the anode to 10.44 near the cathode (A3 and C3, respectively; $p<0.05$). In the anode side, the protons formed by electrolysis were, to some extent, counteracted by the clay soil, potentially with a high cation exchange capacity thus contributing to the buffer capacity around the anode (keeping the pH more stable comparing to the cathode side) possibly due to a high carbonate content [30].

The EK treatment promoted only slight variations of the soil conductivity, mainly attributed to the formation of ions and to the pH changes reported above. The only significant changes were observed in the EK-Abiotic and EK-Biotic between soil sections at the same depth: conductivity in A2 > C2 and A3 > C3 (p<0.05), being mostly related with the higher pH in cathode soil sections (Table 1). At higher pH the precipitation of ions occurs, thus decreasing soil conductivity [31].

Despite the EK application, the temperature of the soil was kept somewhat constant along the 4 days, 23.5 ± 0.5 ºC. The lower water content was always observed in the topsoil layer (C1 and A1 sections) attributed to water evaporation (Table 1), which caused the topsoil layers to crack in the experiments without periodic irrigation. When EK was applied to the soil, differences were also observed in the water content between the anode and the cathode side, for example, the EK-Abiotic and EK-Biotic anode side presented always lower values compared to the cathode side (A1 < C1, A2 < C2 and A3 < C3; p<0.05). The microcosms system was not significantly affected by the ohmic heating as the measured soil temperature was fairly constant along the EK treatment. In agreement, we concluded that the higher water content in the cathode side of the experiments without daily irrigation was due to the electroosmotic flow towards cathode. This behavior was also observed in the experiments with periodic irrigation at a much lower extent (EK-Abiotic-W and EK-Biotic-W; p<0.05). These results are supporting the previously reported by Guedes *et al.* (2019) for the same clay soil, where the electroosmotic flow was developing towards the cathode side [19].

The voltage-drop between the working electrodes greatly increased along time, being more pronounced in the experiments without periodic irrigation: in EK-Biotic from 9.3 to 21.3 V, in EK Abiotic from 7.5 to 90.2 V, in EK-Abiotic-W from 7.8 to 11.1 V and in EK-Biotic-W from 7.6 to 10.1 V. The voltage drop increase is attributed to the depletion of ions (through migration and precipitation reactions), the decreased of water content along time (Table 1), soil cracking and the accumulation of gas at the electrode-soil interface which increases the electrical resistance [32].

3.1.2.CECs Abiotic or Biotic decay under control conditions (without EK)

In the Abiotic microcosm, the CECs total mass loss was below 7% (sum of all CECs mass in relation to total initial value; Figure 2). A higher mass loss, approx. 20% (statically significantly different, p<0.05), was obtained in the Biotic microcosm. The estimated Henry's Law constant for SMX and IBU

are $6.4x10^{-13}$ and $1.5x10^{-7}$ atm-cu m/mole, respectively, the lowest and the highest values among the tested compounds. Accordingly, the CECs decay through volatilization from the moist soil surface is unlikely, or negligible, due to their low vapor pressures. Furthermore, since experiments were not performed under direct UV radiation, a concentration decrease as a result of photodegradation should not be relevant. Thus, it can be inferred that under Abiotic conditions, the decay of CECs observed should be mostly due to aging and sequestration processes [33] and, to some extent, due to analytical extraction efficiencies (ESI 4). Nonetheless, the changes in the soil physicochemical properties caused by the sterilization, due to autoclaving, cannot be disregarded in the comparison between systems as it can affect sorption or sequestration [34]. Herein, the observed CECs abiotic decay levels were below 19% (corresponding to E2) following the order E2 ≥ SMX ≥ BPA ≥ TCS ≈ DCF ≈ IBU ≥ EE2 ≈ OXY, CAF, CBZ.

In the Biotic conditions, the observed decay values should be related to the biodegradation of the CECS, consistent with previous reports [19,35,36]. To systematize our results, we grouped the tested CECs accordingly to the observed Biotic decay levels after 4 days as follows: > 20% were considered biodegradable (E2, SMX, BPA and IBU), and < 20% recalcitrant (EE2, OXY, DCF, TCS, CAF and CBZ) (Figure 3). The observed trend for their Biotic degradation was E2 ≫ SMX ≥ BPA ≈ IBU > EE2 > OXY ≈ DCF ≈ TCS ≈ CAF ≈ CBZ. The highest Biotic degradation was 90% for E2, with the remaining biodegradable CECs (Figure 3a) having decay values between 36.1 and 24.8%. For the recalcitrant CECs (Figure 3b), the Biotic degradation levels were below 13% and, except for EE2, no statistically significant differences were found compared to the Abiotic conditions (p>0.05).

Overall, these results are in accordance with the findings reported in the literature. The average $t_{1/2}$ of BPA (biodegradable) increases in 0.75 times when a non-sterilized soil was used [37]. In a 63-day study, CBZ and TCS (recalcitrant) average $t_{1/2}$ in sterilized soils was 1.6 and 1.7 times longer, respectively [37]. Each CEC will display an unique half-life that is dependent on compounds properties and the environmental conditions [38]. For example, E2, a natural estrogen, is highly susceptible to biodegradation, whereas EE2, a synthetic derivative of E2, is less susceptible due to the presence of an ethynyl group [39]. Consistently, we observed a decay difference of almost 80% between the two estrogens (Figure 3; p<0.05). CBZ (recalcitrant) shows high persistence in soils due to its stable heterocyclic structure, with an estimated $t_{1/2}$ ranging from 28.0 to 39.1 days [37], thus

considered one of the most persistent pharmaceuticals in the terrestrial and aquatic environments. Besides the heterocyclic structure, the presence of halogens negatively affects biodegradation, an effect that rises with an increased degree of halogenation [40]. For example, the degradation of DCF that has two chlorines, and TCS that has 3 chlorines were below 3%. The CECs that are non-ionic, like CAF, have been considered recalcitrant, whereas weak acidic CECs, such as SMX and IBU, exhibit usually fast degradation rates [41,42].

The HPLC-DAD-FLD chromatograms of the treated soil extracts (spiked compared to soil treated without spiking) presented new peaks possibly sub-products and/or biodegradation intermediates of the CECs, that were classified as unknown compounds. Their identification using LC-ESI-MS was not possible under the MS system (single quadrupole; low resolution mass spectrometry) and operational conditions (full scan; positive and negative ion mode; non-targeted analysis) used. To achieve their identification another mass analyzer configuration and high-resolution mass spectrometry or experiments with higher spiking concentration may be required. Still, and considering that some CECs presented good decays in 4 days, it should me mentioned that CECs metabolites can, sometimes, be more harmful that the parent compound. For example, it has been reported that methyl triclosan (methyl-TCS) [43], the main metabolite of TCS, is more persistent in soil [44] and more lipophilic, thus exhibiting higher bioaccumulation potential [45]. Thus, a decrease in the parent CEC concentration does not necessarily mean a decrease of the associated environmental risks.

3.1.3.CECs Abiotic or Biotic decay under EK conditions – general analysis

When EK was applied to the soil, higher CECs decay levels were, in general, achieved compared to the Biotic and Abiotic control conditions (Figure 3). The application of EK in the Abiotic system increased the decay of the recalcitrant CECs between 6 and 21%, and of the biodegradable CECs between 14 and 28% compared to the corresponding control (statistically significantly different for all CECs except for DCF and for CBZ, $p<0.05$). This resulted in a decay trend different for that of the Abiotic control, as follows: SMX > E2 > BPA ≥ TCS ≥ CAF ≈ IBU ≈ EE2 ≥ DCF ≥ OXY ≈ CBZ**.** When EK was combined with bioremediation (*i.e.* biotic conditions), in general, the decay trend observed was similar to that observed in the corresponding control, but the decay levels increased up to 17% ($p<0.05$) for SMX, BPA, DCF, TCS, and CAF.

The use of daily irrigation, which slightly altered the decay order of the CECs compared to the controls, resulted in the highest decay values for all CECs, with EK-Biotic-W retrieving higher or equal decays compared to EK-Abiotic-W, except for SMX (~40% higher in EK-Abiotic-W; $p<0.05$). Statistically significant differences ($p<0.05$) were obtained in the decay levels for all compounds, except for E2 and for CAF, comparing the two EK treatment with and without irrigation.

The CECs spatial distribution in the soil showed that, independently of the experimental setup, at the fourth day their residual amounts were mainly present in the topsoil layer (up to 62%; A1 and C1), and at very low amounts in the soil layer below (up to 6.5%, A2 and C2) (Table 2). The observed distribution is consistent with the spiking of the soil on the topsoil layer that simulates a conventional irrigation system. Due to the hydraulic mobility promoted by the water, the CECs reached the second soil deep (2-4 cm depth, A2 and C2), but the high clay content limited further their migration (4-6 cm depth, A3 and C3). The application of EK resulted, in general, in higher percentage of CECs being detected in the cathode side compared to the anode (Table 2). When no daily irrigation was performed, the cathode side presented CECs between 45 and 62%, and the anode between 38 and 48% (Table 2)

The application of daily irrigation resulted in a more even distribution of the CECs between anode and cathode sides, with some compounds found at higher amounts on the anode side (IBU in EK-Biotic-W and EK-Abiotic-W; SMX and DCF in EK-Abiotic-W), regardless of relative standard deviations (RSD) up to 22%. The uneven distribution of the CECs was attributed to soil matrix heterogeneity, known to largely affect the electromigration and the electroosmotic flow [46], and to the manual pressing, irrigation and fractionation of the soil. Still, and independently of these RSDs, CECs total decay retrieved lower RSDs (< 10%).

3.1.4.CECs decay under EK conditions – compound specific analysis

The comparison of the EK-Biotic with the EK-Abiotic made apparent that the decay of the recalcitrant CECs (EE2, OXY, DCF, TCS, CAF and CBZ) in 4 days was mostly ensured by the application of the EK (*i.e.* their biodegradation is negligent). Also considering the degradation levels observed in the controls (Figure 3, Biotic system), we can conclude that whenever EK is applied the recalcitrant CECs underwent mostly electro-degradation processes. Our experimental results support that EK promoted their degradation through induced redox reactions both close and far away from the

electrodes, the last since the clay particles can act as micro-electrodes [19,46]. It is known that the electro-chemical oxidation of organic contaminants comprises two mechanisms: (i) direct oxidation occurring at the anode surface by the chemisorbed •OH (E_0 = 2.8V), when an active electrode is used (similar to the one used here), and (ii) indirect oxidation through the generation of a mediator in the bulk solution (like active chlorine). The differences observed in the decay levels of the recalcitrant CECs might be explained by their structural differences [47], and/or distinct physicochemical properties (ESI 1). For example, in the 4-aminosalicylic acid the presence of a $–NH_2$ group in *meta*-position to –OH and *para*-position to –COOH slightly activated the electrophilic attack of •OH, compared to the salicylic acid without any $–NH_2$ group, in water samples using a boron doped diamond/air-diffusion cell [47]. In contrast, the electrophilic reaction of •OH was strongly deactivated when the $–NH_2$ group was placed in *para*-position in respect to –OH and *meta*-position in relation to –COOH in the 5-aminosalicylic acid molecule [47].

At this stage we did not aim to disclose the electro-degradation pathway of the tested CECs. Nonetheless, several studies conducted in water matrices have shown that, for example, the electro-chemical degradation of E2 and EE2 are likely initiated by the addition of •OH radicals either through (i) abstraction of the hydrogen linked to the C6 of the aliphatic ring or (ii) oxidation of its hydroxyl group, regardless that in EE2 the presence of the ethinyl group stabilizes the phenolic ring, increasing its resistance to degradation [48,49]. Consistently, we observed that the EE2 presented lower electro-degradations than E2 (p<0.05). However, several soils constituents may reduce CECs electro-degradation, namely the presence of radical scavengers, such as humic acids [50,51], other ions like HCO_3^- and Cl^- [51,52], and pH [50–52]. To date, the majority of the studies on the degradation of CECs were conducted in water matrices, and data on their degradation through radicals produced by advanced oxidation processes, including electro-chemical processes, in soils are scarce. In this study, the HPLC analysis of the soil extracts, before and after the application of the EK treatments, revealed the presence of unknown compounds that formed throughout the process. We hypothesize that these compounds may constitute degradation intermediates of the CECs, and we will seek their identification in the near future.

When EK is applied, CECs decay is also influenced by their mobilization in the soil column due to electroosmosis and electromigration, both greatly affected by pH dependent CECs speciation. The higher CECs decays observed in the irrigated systems is thus explained by their increased

electro-oxidation rates and solubilization due to a higher water content, as well as, due to their speciation that enhances mobilization by electroosmotic flow and electromigration (ionic species). In this case, we cannot disregard that the soil sterilization may have changed its structure, as the heat and the pressure achieved during autoclaving may increase the surface area available for the sorption of CECs [34], thus negatively affecting their mobilization through electroosmosis and electromigration. On the other hand, the autoclaving of the soil can also increase the dissolved organic content in the soil liquid phase, which can decrease the sorption of the CECs due to complexation in the liquid phase [34], which may actually improve their mobilization through the soil column. All these factors need to be accounted when comparing the mobilization of the CECs in the Biotic and Abiotic system. Still, no significant changes were observed in the pH and conductivity after the sterilization (Table 1) and remained above 7 in all experiments after the 4 days. Thus, considering that the pKa of IBU is 4.91 (ESI 1), it can dissociate in its anionic form and potentially move towards the anode side by electromigration, and towards the cathode by electroosmotic flow. Higher IBU concentrations were detected in the anode side (Table 2) compared to the cathode (p<0.05) in the EK-Biotic-W and EK-Abiotic-W experiments, which led to the highest observed decays for IBU. This suggests that, in this case, electromigration prevailed in relation to electroosmosis. CAF presented a homogenous distribution in the soil (Table 2; p>0.05), hence most probably suffered electro-degradation after its desorption from the soil particles (*e.g.* desorption coefficient logarithm is 2.87 and 3.89 for silt and sandy loam soils, respectively) promoted by the OFF-time period [19]. On the other hand, TCS removal by EK was shown to be pH dependent [19], thus a pH above its pKa (Table 2; pKa of 7.9, ESI 1), promoted by the water electrolysis when EK was applied, favors its solubilization and movement toward the electrodes, consequently, its degradation (Figure 3).

EK application may enhance bioremediation due to higher availability of the CECs, nutrients and electron acceptors and can also promote bacteria mobility along the soil due to electrophoresis and electroosmotic flow [14], favoring the contact between bacteria and the contaminants. In addition, EK application generates oxidizing and reducing zones that are favorable for biodegradation of contaminants close to the electrodes [14]. Collectively these factors explain the synergistic effect between EK and bioremediation noticed for the biodegradable CECs (except IBU) that showed higher decays in the EK-Biotic-W compared to EK-Abiotic-W (Figure 3; p<0.05).

The pH shifts promoted by EK may favor the CECs mobilization, however bioremediation can be hindered close to the electrodes [14,53], specially near the cathode side in which pH became alkaline (Table 1). This hypothesis is supported by E2, BPA and EE2 distribution in the soil column of the EK-Biotic systems, with/without daily irrigation, that, in general, accumulated in the cathode side (pH 9), contrary to their homogeneously distribution observed in the EK-Abiotic systems (Table 2). At the end of EK-Biotic-W, EE2 and BPA distribution is 18.2 and 15.6% higher in C1 than in A1 (p<0.05), and E2 is 24.6% higher in C1 than A1 in the EK-Biotic (p<0.05).

SMX is biodegradable, reaching 36% of biotic decay in 4 days (control conditions). Surprisingly, SMX decay in EK-Abiotic-W was 81% (Figure 3), whereas in EK-Biotic with/without irrigation was ~50%. SMX distribution in the soil was different in these conditions, concentrating in the anode in the EK-Abiotic-W (less 17% in C1 than in A1), but in the cathode in the EK-Biotic-W (more 20% in C1 than in A1). We hypothesize that in EK-Abiotic-W the high-water content (Table 1), combined with a potential higher dissolved organic content in the sterilized soil [34], improved SMX solubilization/mobilization, allowing a more efficient electro-oxidation that surpasses the efficiency of bioremediation.

TCS highest decay was observed in the EK-Biotic without irrigation, whereas in the EK-Biotic-W it was 0% (Table 2). TCS biodegradation was reported to be substantially hindered by a high water content in soil [43], which partially explains that we observed higher TCS degradation levels in the EK-Biotic compared to EK-Biotic-W (Table 1). However, it fails to explain why in the EK-Biotic-W, TCS did not undergo electro-degradation compared to the 22% decay observed in the EK-Abiotic-W (Table 2). TCS major biotransformation methyl-TCS [43], may undergo the reversible reaction in soil [44]. Accordingly, we hypothesize that in the EK-Biotic-W the TCS decay was nil because of the reversibility of TCS conversion to methyl-TCS. This hypothesis deserves further analysis.

3.2. *Round 2:* effects of *longer* EK application

3.2.1. Effects of *longer* EK application on soil physicochemical parameters

In round 2 experiments, as the water content highly influenced CECs decays, the microcosms were irrigated every 4 days and the treatment was conducted at room temperature.

In round 2 experiments (4, 8 and 16 days), soil physicochemical properties (Table 3) presented a similar behavior to what was observed in the round 1 experiments (4 days). In general, pH, conductivity and water content did not significantly change in the Biotic conditions (p>0.05). The application of EK resulted in pH variations in depth, being more pronounced in the *longer* time periods (p<0.05), both in the anode and in the cathode side, with pH changing faster in the cathode than in the anode. The cathode pH on day 4 shifted to slightly alkaline (8.94 - 9.53), whereas in the anode the pH decrease was only observed on day 8 (6.40 - 6.61). On day 16 pH ranged from between 4.31 (C3) to 10.51 (A3).

The conductivity also varied in the EK experiments, being the variation linked to pH, conductivity decreased as pH increased. Water content increased with depth and, in general, from anode to cathode (p<0.05), possibly indicating electroosmotic flow towards the cathode. This is in accordance with the observations in round 1 EK experiments (Table 1). The temperature of the soil was kept constant along the 4, 8 and 16 days, 19.0 ± 0.5 ºC. The voltage-drop between the two working electrodes tended to increase along time with the same pattern, by increasing from approx. 9 to 22 V every 4 days, due to microcosms being watered every 4 days.

3.2.2.CECs removal in *longer* treatment time – general analysis

The total mass loss of the CECs was higher in the first 8 days when EK was applied to the soil (p<0.05; ESI 10). The mass loss was 21.7 and 34.6% at the end of 4 and 8 days, respectively, for the Biotic control, and 30.3 and 54.8% for the EK-Biotic treatment. By other words, under the conditions here tested, the usage of EK during the first 8 days increased the overall removal of CECs compared to their natural attenuation. Unexpectedly, we observed an inversion of this trend for *longer* treatment times, specifically between day 8 and 16, where the removal ratio decreased in the EK-Biotic (increased ~10% relative to day 8) compared to that of the Biotic control (increased nearly 30% relative to day 8). At the end of the 16 days, the total mass loss of the CECs was similar (p>0.05; ESI 10) for the Biotic and EK-Biotic treatments, approx. 64 and 65%, respectively.

As expected, CECs decay increased along the treatment period (Figure 4). In round 2, the CECs under study presented 3 types of biodegradation behaviors, considering their decay and half-life ($t_{½}$), after 16 days (Figure 4 and Table 4):

- Type I – decay ≥ 80%, $t_{½}$ ≤ 8 days: E2, SMX and BPA.
- Type II – with 50% ≤ decay < 80%, 8 days ≤ $t_{½}$ < 16 days: IBU, EE2, DCF and CAF.
- Type III – decay ≤ 50%, $t_{½}$ ≥ 16 days: OXY, TCS and CBZ.

The type I CECs presented high biodegradation efficiency in the first 8 days (Figure 4) with E2 reaching 100% decay (not detected in the soil), followed by BPA (72.8%) and SMX (55.7%). Between days 8 and 16, BPA decay reached 91.7% and SMX 86.6%. In the type II and III CECs we observed a *lag* phase due to the microbiota acclimation, which was followed by a biodegradation stage. In type II, decays on day 8 were between 15 and 43%, increasing to 61.1% for EE2, 75.5% for IBU, 68.5% for DCF and 74.1% for CAF on day 16 (Figure 4). Finally, the type III compounds presented decays below 10% on day 8, after which they increased till 35.7% for OXY, 23.1% for TCS and 21.5% for CBZ. The degradation kinetics here obtained accounted 4 experimental data points for each compound (except for E2 that achieved a very fast decay), with determination coefficients (R^2) above 0.98 for type I compounds, hence a good fit to the pseudo-first order kinetics (Table 4). When a *lag* phase was present (types II and III), the determination coefficients were lower for the pseudo first-order kinetic, between 0.83 and 0.95. In these cases, the negative K_2 values are associated with the existence of a *lag* phase or acclimation period at the beginning of the experiments, being very low (≤10^{-5}), thus suggesting that there is a negligible inhibitory effect in the degradation process at the concentrations here tested [29].

The CECs distribution in the soil also varied along time (Table 5). In the Biotic experiment, compounds were more evenly distributed in the top soil layer, apart from E2 and IBU at day 4, and SMX and IBU on day 8 and 16. When the EK treatment was applied, and similarly to the results obtained in the round 1 experiments, the compounds distribution changed after 4 days with the amount of CECs detected in the anode side being in general lower than in the cathode at day 8, after which CECs were again more evenly distributed (Table 5). The exception to this behavior were SMX, IBU and DCF, that were more concentrated in the anode side on day 8 and 16, than in the cathode. As the pH decreases in the anode side and increases in the cathode (Table 3), the percentage of SMX, IBU and DCF increased in the anode. Considering that these CECs pKa are below 5 (all other CECs pKa are above this value), as the pH shifts to near 5 in the anode side, their migration decreases influencing their degradation.

Comparison of the results obtained in both experimental rounds, at 19.0 ºC and at 23.5 ºC, at the 4th day, no significant differences (p>0.05) were observed for the total mass of CECs removed, thus presenting minimal influence on the CECs decays, in the conditions here studied. Still, some CECs presented individually slightly different behaviors (Figure 3 *vs* Figure 4), SMX decay was always higher, after 4 days, at 18 ºC (p<0.05), more 9.0 and 14.1% in the Biotic and EK-Biotic, respectively. BPA natural attenuation was higher at 18 ºC (more 21.7%, p<0.05), whereas for IBU it was lower (less 8.4%, p<0.05). CAF and TCS decay also changed in the EK-Biotic, with CAF decay increasing at 18 ºC (more 12.6%, p<0.05), and TCS decreasing (less 10.1%, p<0.05).

3.2.3.CECs removal in *longer* treatment time – specific analysis

The individual CECs decay was generally stimulated when EK was applied (EK-Biotic *vs* Biotic) till day 8 (p<0.05), but at day 16 the CECs decays were similar to those observed in the Biotic system (control), with a few exceptions (Figure 4). Regarding the type I compounds (decay ≥ 80%, $t_{½}$ ≤ 8 days), the application of EK could only improve SMX removal, with this antibiotic always presenting higher decays in the EK-Biotic compared to the Biotic (p<0.05). Still, the positive effect was only seen till day 8, after which the EK-Biotic removal of SMX was almost null (0.3%), whereas it increased ~40% in the Biotic system. Among the type II compounds (with 50% ≤ decay < 80%, 8 days ≤ $t_{½}$ < 16 days), EK had a positive impact on all CECs decays till day 8, except on IBU. Decay levels achieved for CAF, DCF and EE2 were always higher on EK-Biotic (p<0.05) till day 8, after which their decay slowed down. This resulted in similar decays for CAF and EE2 after 16 days with and without EK (p>0.05), with DCF reaching better results in the Biotic system (p<0.05). IBU was negatively affected by EK, an effect more pronounced along time, with the EK-Biotic system leading to significantly lower removals on days 8 and 16 comparing to the Biotic system, -9.1 and -21.6%, respectively (p<0.05). Finally, the decay of the compounds grouped in the type III (decay ≤ 50%, $t_{½}$ ≥ 16 days) was positively impacted by EK till day 16. In this group decays were always higher in the EK-Biotic treatment (p<0.05; except for TCS on day 16). Specifically, EK allowed to revoke the *lag* phase as, *e.g.,* on day 4, TCS and OXY showed degradation values of 7.3 and 14.8% when EK was applied, compared to a null decay on the Biotic treatment (Figure 4). The increased removal of CECs in the anode might be due to electro-oxidation processes, but also to an increased bioremediation efficiency as referred before. It has been suggested that the oxygen generated at the anode by electrolysis can increase

dehydrogenase activity and oxygen uptake rate [54,55]. Also, if a pH close to neutral is maintained in the sub-surface, the EK process can increase the interaction between bacteria and the contaminants, and rate limiting nutrients, which improves bioremediation [56].

The decreased CECs decay after day 8 in the EK-Biotic comparing to the Biotic treatment, is partially explained by a decrease in the biodegradation contribution due to community inhibition promoted by the changes promoted in the soil pH (Table 3) but also in the soil redox potential, gas composition and nutrients depletion in the vicinity of the electrodes (due to migration). For example, if pH changes to values below 3 and above 9 (as observed in the cathode side; Table 3), the microbial growth may be inhibited due to interferences with microbial metabolism, gas solubility and nutrients (bio)availability in the pore fluid [54,57,58], reducing biodegradation efficiency. Also, the oxygen generated at the anode may diffuse through the soil thus changing redox potential which may impact both abiotic and biotic transformation [59]. Nonetheless, as seen by the results obtained in the round 1 EK-Abiotic assay (Figure 3), the CECs should have, to some extent, undergone electro-chemical induced degradation. Therefore, the low electro-chemical degradations obtained can be due to (i) decreased CECs solubilization as the pH decreases in the anode side; (ii) decreased electro-osmotic flow. Also, Fenton-*like* reactions leading to the production of •OH radicals that participate in the CECs electro-degradation may have been hindered as the pH increases in the cathode side. The pH increase promotes soluble Fe species precipitation, the Fe^{3+} decreases as the pH increases [53].

Further analyses are still required to understand the mechanism that reduced the overall efficiency of electro-chemical degradation between days 8 and 16.

Overall, under the conditions here tested, we conclude that the benefits of the EK application are hindered when *longer* periods are used. This likely results from the alteration of the soil properties, namely pH, that negatively impacts the efficiency of bioremediation and of electro-degradation near the cathode. To circumvent this effect there are several possible routes, for example, the EK treatment may be interrupted, a polarization reversal can be used after day 8, or additives can be employed during irrigation to minimize the pH changes. Our results reveal that the complexity of CECs mixture used here, combining biodegradable and recalcitrant compounds of diverse chemicals structures, as a representation of a common RWW, dramatically increased the complexity of the analysis of the EK assisted remediation treatment. Still, further studies are needed to better understand the

physicochemical processes involved in the CECs removal, their degradation pathways and the microbiota changes induced by EK. Understanding these factors, and the complexity of their interactions, is required to reach a knowledge-based strategy for operational optimization and control of EK application in the field.

4. Conclusions

In the sterile soil, the observed Abiotic decay after 4 days accounted for a reduction of the total mass loss of CECs below 7%. This value increased to 20% when Biotic conditions were used, with decay levels for individual compounds varying from 13% (recalcitrant CECs) to 90% (E2, the most biodegradable compound). In general, the application of the EK treatment for four days resulted in higher decay levels, in both Biotic (ca. 17% increase) and Abiotic conditions (6 to 28% increases). Interestingly, the benefit of EK application for 4 days was more obvious for the recalcitrant compounds OXY, DCF, TCS, CAF and CBZ, regardless of the presence of the soil microbiota, showing that they mostly undergo electro-chemical induced degradation. The use of daily irrigation during the four days' EK treatment resulted in the highest decay values for all CECs (except SMX) due to higher electro-chemical oxidation rates and compound's mobilization by electroosmosis and electromigration. The conclusions established in these *short-term*-treatments (round 1) were challenged when *longer* periods of treatment were used (round 2). In fact, overall, the results obtained for the *longer* experiments (round 2) results support the application of EK to accelerate the CECs removal in the first 8 days, after which *longer* EK application (20 mA, ON/OFF periods of 12 h) negatively impacted the removal of most CECs. The exception are the compounds classified as type III – decay ≤ 50%, $t_{½}$ ≥ 16 days, were EK positively increased their decay levels, even after 16 days of treatment.

We conclude that, in the here tested conditions, the EK assisted remediation should be applied for *short* periods and its design should be guided by the irrigation regime used, since we observed that the water content influences the electro-degradation of some CECs. In addition, the selection of the EK conditions should definitively target the most recalcitrant compounds, yet they also need to ensure that the pace of the bioremediation processes is not negatively affected.

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References

[1] FAO, Voluntary Guidelines for Sustainable Soil Management, Voluntary Guidelines for Sustainable Soil Management Food and Agriculture Organization of the United Nations Rome, Italy, 2017.

[2] M. Biel-Maeso, C. Corada-Fernández, P.A. Lara-Martín, Monitoring the occurrence of pharmaceuticals in soils irrigated with reclaimed wastewater, Environ. Pollut. 235 (2018) 312–321. doi:10.1016/j.envpol.2017.12.085.

[3] European Commission, Proposal for a Regulation of the European Parliament and of the Council on Minimum Requirements for Water Reuse (COM(2018)337), 2018.

[4] Decreto-Lei n.º 119/2019, n.d.

[5] (UE) 2020/741, (n.d.) I 177/32-55.

[6] M. Pan, L.M. Chu, Fate of antibiotics in soil and their uptake by edible crops, Sci. Total Environ. 599–600 (2017) 500–512. doi:10.1016/j.scitotenv.2017.04.214.

[7] D. Azanu, C. Mortey, G. Darko, J.J. Weisser, B. Styrishave, R.C. Abaidoo, Uptake of antibiotics from irrigation water by plants, Chemosphere. 157 (2016) 107–114. doi:10.1016/j.chemosphere.2016.05.035.

[8] N. Gottschall, E. Topp, C. Metcalfe, M. Edwards, M. Payne, S. Kleywegt, P. Russell, D.R. Lapen, Pharmaceutical and personal care products in groundwater, subsurface drainage, soil, and wheat grain, following a high single application of municipal biosolids to a field, Chemosphere. 87 (2012) 194–203. doi:10.1016/j.chemosphere.2011.12.018.

[9] Y. Wang, J. Lu, L. Mao, J. Li, Z. Yuan, P.L. Bond, J. Guo, Antiepileptic drug carbamazepine promotes horizontal transfer of plasmid-borne multi-antibiotic resistance genes within and across bacterial genera, ISME J. 13 (2019) 509–522. doi:10.1038/s41396-018-0275-x.

[10] J. Lu, M. Jin, S.H. Nguyen, L. Mao, J. Li, L.J.M. Coin, Z. Yuan, J. Guo, Non-antibiotic antimicrobial triclosan induces multiple antibiotic resistance through genetic mutation, Environ. Int. 118 (2018) 257–265. doi:10.1016/j.envint.2018.06.004.

[11] Y. Shen, R.D. Stedtfeld, X. Guo, G.D. Bhalsod, S. Jeon, J.M. Tiedje, H. Li, W. Zhang, Pharmaceutical exposure changed antibiotic resistance genes and bacterial communities in soil-surface- and overhead-irrigated greenhouse lettuce, Environ. Int. 131 (2019) 105031. doi:10.1016/j.envint.2019.105031.

[12] J. Tang, C. Zhang, X. Shi, J. Sun, J.A. Cunningham, Municipal wastewater treatment plants coupled with electrochemical, biological and bio-electrochemical technologies: Opportunities and challenge toward energy self-sufficiency, J. Environ. Manage. 234 (2019) 396–403. doi:10.1016/j.jenvman.2018.12.097.

[13] P. Singh, V.K. Singh, R. Singh, A. Borthakur, S. Madhav, A. Ahamad, A. Kumar, D.B. Pal, D. Tiwary, P.K. Mishra, Bioremediation: A sustainable approach for management of environmental contaminants, Elsevier Inc., 2019. doi:10.1016/B978-0-12-818095-2.00001-1.

[14] R.T. Gill, M.J. Harbottle, J.W.N. Smith, S.F. Thornton, Electrokinetic-enhanced bioremediation of organic contaminants: A review of processes and environmental applications, Chemosphere. 107 (2014) 31–42. doi:10.1016/j.chemosphere.2014.03.019.

[15] 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.

[16] K.R. Reddy, C. Cameselle, Overview of electrochemical remediation technologies, in: K.R. Reddy, C. Cameselle (Eds.), Electrochem. Remediat. Technol. Polluted Soils, Sediments Groundw., Wiley, 2009.

[17] A.R. Ferreira, P. Guedes, E.P. Mateus, A.B. Ribeiro, N. Couto, Emerging organic contaminants in soil irrigated with effluent: electrochemical technology as a remediation strategy, Sci. Total Environ. 743 (2020) 140544. doi:10.1016/j.scitotenv.2020.140544.

[18] Y.B. Acar, A.N. Alshawabkeh, Principles of Electrokinetic Remediation, Environ. Sci. Technol. 27 (1993) 2638–2647. doi:10.1021/es00049a002.

[19] P. Guedes, V. Lopes, N. Couto, E.P. Mateus, C.S. Pereira, A.B. Ribeiro, Electrokinetic remediation of contaminants of emergent concern in clay soil: Effect of operating parameters, Environ. Pollut. 253 (2019) 625–635. doi:10.1016/j.envpol.2019.07.040.

[20] L.Y. Wick, L. Shi, H. Harms, Electro-bioremediation of hydrophobic organic soil-contaminants: A review of fundamental interactions, Electrochim. Acta. 52 (2007) 3441–3448. doi:10.1016/j.electacta.2006.03.117.

[21] H. Li, B. Li, J. Ma, J. Ye, P. Guo, L. Li, Fate of antibiotic-resistant bacteria and antibiotic resistance genes in the electrokinetic treatment of antibiotic-polluted soil, Chem. Eng. J. 337 (2018) 584–594. doi:10.1016/j.cej.2017.12.154.

[22] C. Cameselle, K.R. Reddy, Development and enhancement of electro-osmotic flow for the removal of contaminants from soils, Electrochim. Acta. 86 (2012) 10–22. doi:10.1016/j.electacta.2012.06.121.

[23] N. Ratola, A. Cincinelli, A. Alves, A. Katsoyiannis, Occurrence of organic microcontaminants in the wastewater treatment process. A mini review, J. Hazard. Mater. 239–240 (2012) 1–18. doi:10.1016/j.jhazmat.2012.05.040.

[24] A. Bielen, A. Šimatović, J. Kosić-Vukšić, I. Senta, M. Ahel, S. Babić, T. Jurina, J.J. González

Plaza, M. Milaković, N. Udiković-Kolić, Negative environmental impacts of antibiotic-contaminated effluents from pharmaceutical industries, Water Res. 126 (2017) 79–87. doi:10.1016/j.watres.2017.09.019.

[25] P. Paíga, M. Correia, M.J. Fernandes, A. Silva, M. Carvalho, J. Vieira, S. Jorge, J.G. Silva, C. Freire, C. Delerue-Matos, Assessment of 83 pharmaceuticals in WWTP influent and effluent samples by UHPLC-MS/MS: Hourly variation, Sci. Total Environ. 648 (2019) 582–600. doi:10.1016/j.scitotenv.2018.08.129.

[26] M. Shestakova, M. Sillanpää, Electrode materials used for electrochemical oxidation of organic compounds in wastewater, Rev. Environ. Sci. Biotechnol. 16 (2017) 223–238. doi:10.1007/s11157-017-9426-1.

[27] C. Yuan, C.Y. Chen, C.H. Hung, Electrochemical remediation of BPA in a soil matrix by Pd/Ti and RuO 2/Ti electrodes, J. Appl. Electrochem. 43 (2013) 1163–1174. doi:10.1007/s10800-013-0600-z.

[28] R.M. Baena-Nogueras, E. González-Mazo, P.A. Lara-Martín, Degradation kinetics of pharmaceuticals and personal care products in surface waters: photolysis vs biodegradation, Sci. Total Environ. 590–591 (2017) 643–654. doi:10.1016/j.scitotenv.2017.03.015.

[29] E. Pérez-Carrera, V.M. León, P.A. Lara-Martín, E. González-Mazo, Influence of the hydrophilic moiety of anionic and nonionic surfactants on their aerobic biodegradation in seawater, Sci. Total Environ. 408 (2010) 922–930. doi:10.1016/j.scitotenv.2009.10.003.

[30] K.R. Reddy, U.S. Parupudi, S.N. Devulapalli, C.Y. Xu, Effects of soil composition on the removal of chromium by electrokinetics, J. Hazard. Mater. 55 (1997) 135–158. doi:10.1016/S0304-3894(97)00020-4.

[31] K.R. Reddy, K. Darko-Kagya, A.Z. Al-Hamdan, Electrokinetic Remediation of Chlorinated Aromatic and Nitroaromatic Organic Contaminants in Clay Soil, Environ. Eng. Sci. 28 (2011) 405–413. doi:10.1089/ees.2010.0235.

[32] E.C. Lee, Electrokinetic Improvement of Soft Clay Using Electrical Vertical Drains, Elsevier

Journal Pre-proof

Ltd., 2015. doi:10.1016/B978-0-08-100191-2.00015-0.

[33] F. Liu, G.G. Ying, J.F. Yang, L.J. Zhou, R. Tao, L. Wang, L.J. Zhang, P.A. Peng, Dissipation of sulfamethoxazole, trimethoprim and tylosin in a soil under aerobic and anoxic conditions, Environ. Chem. 7 (2010) 370–376. doi:10.1071/EN09160.

[34] K. Lees, M. Fitzsimons, J. Snape, A. Tappin, S. Comber, Soil sterilisation methods for use in OECD 106: How effective are they?, Chemosphere. 209 (2018) 61–67. doi:10.1016/j.chemosphere.2018.06.073.

[35] A. Karnjanapiboonwong, A.N. Morse, J.D. Maul, T.A. Anderson, Sorption of estrogens, triclosan, and caffeine in a sandy loam and a silt loam soil, J. Soils Sediments. 10 (2010) 1300–1307. doi:10.1007/s11368-010-0223-5.

[36] J. Xu, L. Wu, A.C. Chang, Degradation and adsorption of selected pharmaceuticals and personal care products (PPCPs) in agricultural soils, Chemosphere. 77 (2009) 1299–1305. doi:10.1016/j.chemosphere.2009.09.063.

[37] Y. Yu, Y. Liu, L. Wu, Sorption and degradation of pharmaceuticals and personal care products (PPCPs) in soils, Environ. Sci. Pollut. Res. 20 (2013) 4261–4267. doi:10.1007/s11356-012-1442-7.

[38] T. Zhang, B. Wu, N. Sun, Y. Ye, H. Chen, Sorption and degradation of wastewater-associated pharmaceuticals and personal care products in agricultural soils and sediment, Water Sci. Technol. 68 (2013) 991–998. doi:10.2166/wst.2013.326.

[39] B. Stumpe, B. Marschner, Factors controlling the biodegradation of 17β-estradiol, estrone and 17α-ethinylestradiol in different natural soils, Chemosphere. 74 (2009) 556–562. doi:10.1016/j.chemosphere.2008.09.072.

[40] H. Loonen, F. Llndgren, B. Hansen, W. Karcher, J. Nlemela, K. Hiromatsu, M. Takatsuki, W. Peunenburg, E. Rorije, J. Struijs, Prediction of biodegradability from chemical structure: Modeling of ready biodegradation test data, Environ. Toxicol. Chem. 18 (1999) 1763–1768. doi:10.1002/etc.5620180822.

Journal Pre-proof

[41] A.J. Al-Rajab, L. Sabourin, D.R. Lapen, E. Topp, The non-steroidal anti-inflammatory drug diclofenac is readily biodegradable in agricultural soils, Sci. Total Environ. 409 (2010) 78–82. doi:10.1016/j.scitotenv.2010.09.020.

[42] A. Grossberger, Y. Hadar, T. Borch, B. Chefetz, Biodegradability of pharmaceutical compounds in agricultural soils irrigated with treated wastewater, Environ. Pollut. 185 (2014) 168–177. doi:10.1016/j.envpol.2013.10.038.

[43] E. Butler, M.J. Whelan, R. Sakrabani, R. Van Egmond, Fate of triclosan in fi eld soils receiving sewage sludge, Environ. Pollut. 167 (2012) 101–109. doi:10.1016/j.envpol.2012.03.036.

[44] N. Lozano, C.P. Rice, M. Ramirez, A. Torrents, Fate of Triclosan and Methyltriclosan in soil from biosolids application, 160 (2012) 103–108. doi:10.1016/j.envpol.2011.09.020.

[45] A. Macherius, D.R. Lapen, T. Reemtsma, J. Römbke, E. Topp, A. Coors, Triclocarban, triclosan and its transformation product methyl triclosan in native earthworm species four years after a commercial-scale biosolids application, Sci. Total Environ. 472 (2014) 235–238. doi:10.1016/j.scitotenv.2013.10.113.

[46] S. Pamukcu, Electrochemical Transport and Transformations, Electrochem. Remediat. Technol. Polluted Soils, Sediments Groundw. (2009) 29–64. doi:10.1002/9780470523650.ch2.

[47] X. Florenza, S. Garcia-Segura, F. Centellas, E. Brillas, Comparative electrochemical degradation of salicylic and aminosalicylic acids: Influence of functional groups on decay kinetics and mineralization, Chemosphere. 154 (2016) 171–178. doi:10.1016/j.chemosphere.2016.03.112.

[48] N. Xu, Y. Zhang, H. Tao, S. Zhou, Y. Zeng, Bio-electro-Fenton system for enhanced estrogens degradation, Bioresour. Technol. 138 (2013) 136–140. doi:10.1016/j.biortech.2013.03.157.

[49] P. Guedes, E.P. Mateus, N. Couto, Y. Rodríguez, A.B. Ribeiro, Electrokinetic remediation of six emerging organic contaminants from soil, Chemosphere. 117 (2014) 124–131. doi:10.1016/j.chemosphere.2014.06.017.

[50] Y. Liu, Y. Zhang, B. Wang, S. Wang, M. Liu, Y. Wu, L. Lu, H. Ren, H. Li, W. Dong, A. Qadeer,

Degradation of ibuprofen in soil systems by persulfate activated with pyrophosphate chelated Fe(II), Chem. Eng. J. 379 (2020) 122145. doi:10.1016/j.cej.2019.122145.

[51] Y. Liu, S. Wang, Y. Wu, H. Chen, Y. Shi, M. Liu, W. Dong, Degradation of ibuprofen by thermally activated persulfate in soil systems, Chem. Eng. J. 356 (2019) 799–810. doi:10.1016/j.cej.2018.09.002.

[52] Amina, X. Si, K. Wu, Y. Si, B. Yousaf, Mechanistic insights into the reactive radicals-assisted degradation of sulfamethoxazole via calcium peroxide activation by manganese-incorporated iron oxide–graphene nanocomposite: Formation of radicals and degradation pathway, Chem. Eng. J. 384 (2020) 123360. doi:10.1016/j.cej.2019.123360.

[53] A.T. Yeung, Y.Y. Gu, A review on techniques to enhance electrochemical remediation of contaminated soils, J. Hazard. Mater. 195 (2011) 11–29. doi:10.1016/j.jhazmat.2011.08.047.

[54] S.H. Kim, H.Y. Han, Y.J. Lee, C.W. Kim, J.W. Yang, Effect of electrokinetic remediation on indigenous microbial activity and community within diesel contaminated soil, Sci. Total Environ. 408 (2010) 3162–3168. doi:10.1016/j.scitotenv.2010.03.038.

[55] P. She, B. Song, X.H. Xing, M. Van Loosdrecht, Z. Liu, Electrolytic stimulation of bacteria Enterobacter dissolvens by a direct current, Biochem. Eng. J. 28 (2006) 23–29. doi:10.1016/j.bej.2005.08.033.

[56] A. Saini, D.N. Bekele, S. Chadalavada, C. Fang, R. Naidu, A review of electrokinetically enhanced bioremediation technologies for PHs, J. Environ. Sci. (China). 88 (2020) 31–45. doi:10.1016/j.jes.2019.08.010.

[57] E. Mena, J. Villaseñor, P. Cañizares, M.A. Rodrigo, Effect of electric field on the performance of soil electro-bioremediation with a periodic polarity reversal strategy, Chemosphere. 146 (2016) 300–307. doi:10.1016/j.chemosphere.2015.12.053.

[58] D. Huang, S. Guo, T. Li, B. Wu, Coupling interactions between electrokinetics and bioremediation for pyrene removal from soil under polarity reversal conditions, Clean - Soil, Air, Water. 41 (2013) 383–389. doi:10.1002/clen.201200079.

Journal Pre-proof

[59] A.N. Alshawabkeh, Electrokinetic soil remediation: Challenges and opportunities, Sep. Sci. Technol. 44 (2009) 2171–2187. doi:10.1080/01496390902976681.

Journal Pre-proof

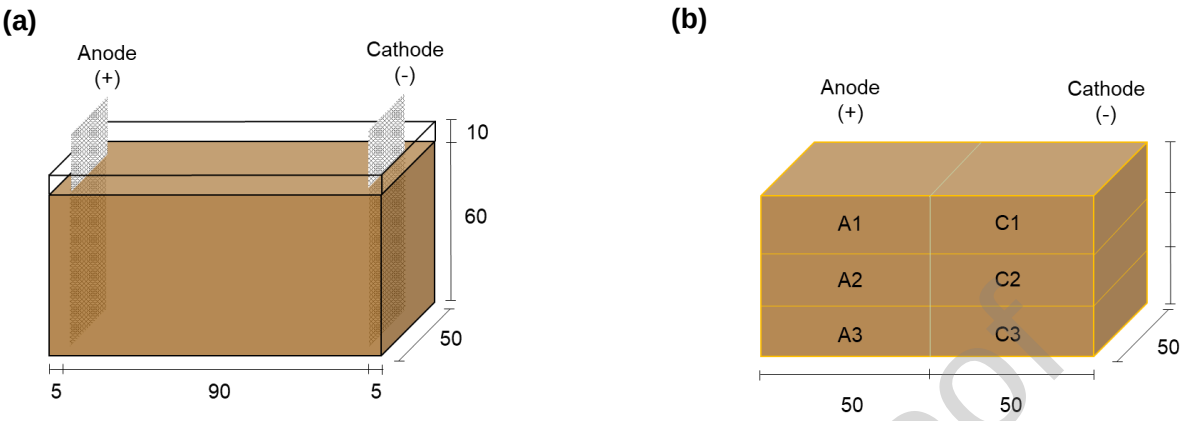

Figure 1. Schematic representation of (a) microcosm and (b) the soil sections collected at the end of the experiments. The light green line represents the tangential cut (anode - A, cathode - C) and the yellow represent the transversal cuts (layers 1, 2 and 3). All units are in mm.

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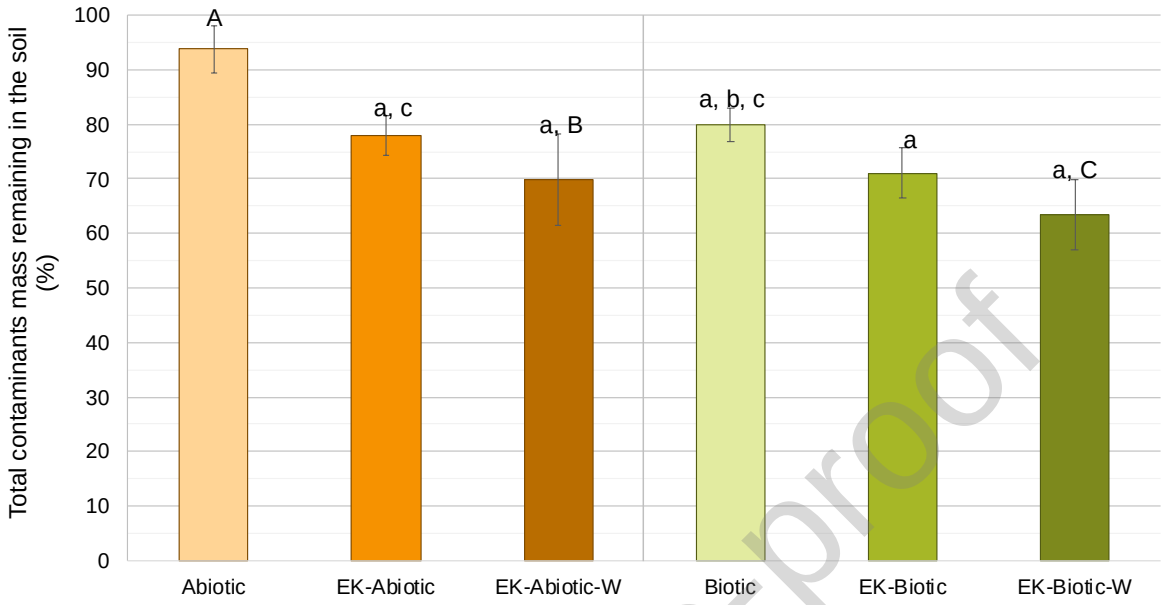

Figure 2. Sum of all CECs mas remaining in the soil after 4 days under the different treatments conducted at 23.5 ºC (round 1; statistical analysis: multiple comparisons were statistically performed at $p<0.05$, 95% confidence interval; data that has capital case letters is statistically significantly different with the accordingly lower letter; n=2)

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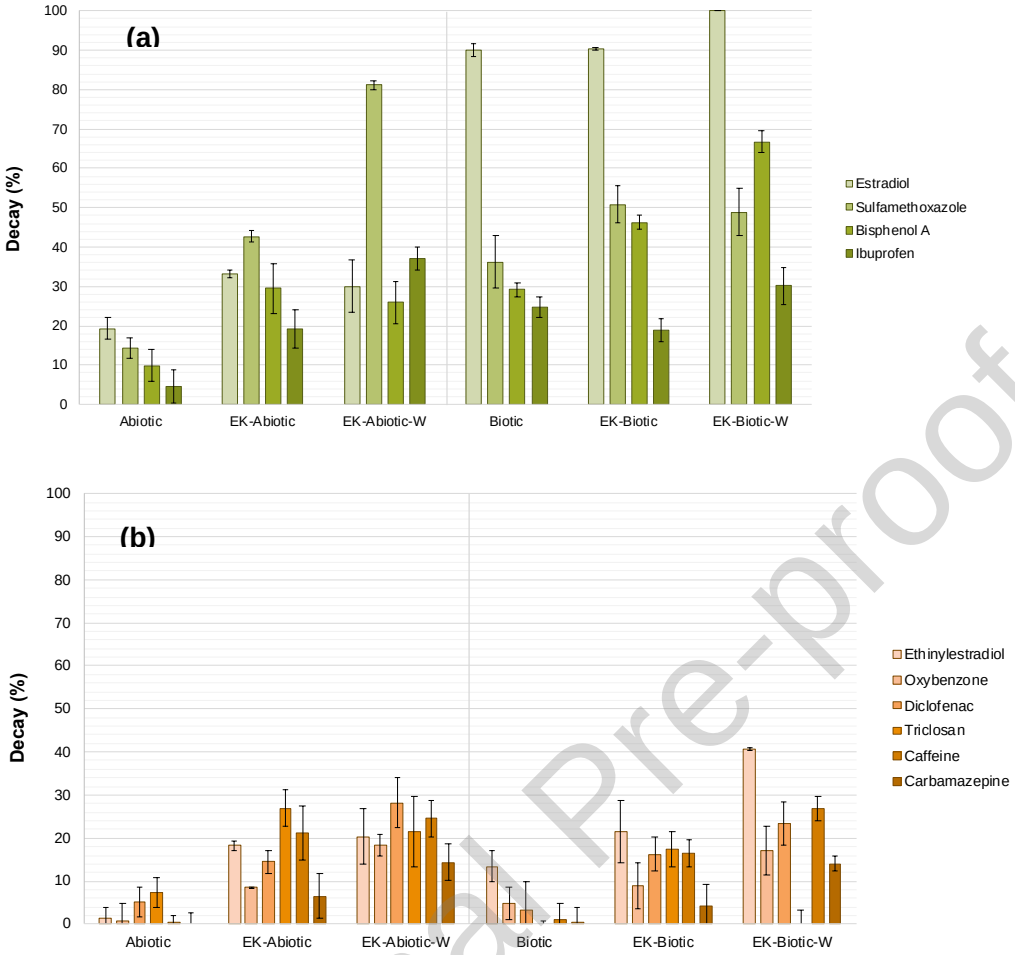

Figure 3. Degradation percentages and standard deviations (mean ± SD) of the (a) biodegradable and (b) recalcitrant organic contaminants for all treatments conducted at 23.5 ºC for 4 days (round 1; statistical data available in ESI 6; n=2).

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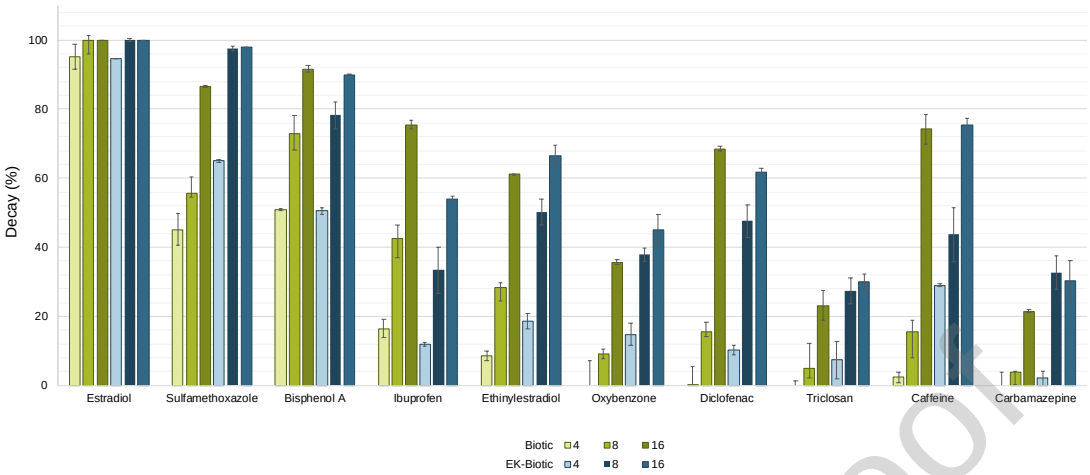

Figure 4. Degradation percentages and standard deviations (mean ± SD) of all contaminants of emergent concern for the Biotic control and EK-Bio treatment conducted for 4, 8 and 16 days at room temperature, 19 ± 0.5 ºC (round 2; statistical data available in ESI 11; n=2).

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Table 1. Soil pH, electric conductivity, water content by soil section for the round 1 experiments conducted for 4 days (average soil temperature 23.5 ± 0.5 ºC). (standard deviations not shown for simplicity; available in ESI 5; n=2).

Soil		Experiment					
Section	**Layer**	**Abiotic**	**EK-Abiotic**	**EK-Abiotic-W**	**Biotic**	**EK-Biotic**	**EK-Biotic-W**
pH							
Anode	A1	7.84	7.81 ^{c}	7.28 ^{j}	7.89	7.75 ^{g}	7.59 ^{O}
	A2	8.07 ^{a}	7.03 ^{A,d}	7.40 ^{k}	8.05 ^{a}	7.34 ^{h}	7.43 ^{p}
	A3	8.02 ^{b,f}	6.94 ^{B,e}	7.32 ^{m}	8.14 ^{b,f,n}	7.11 ^{F,i}	7.25 ^{N,q}
Cathode	C1	7.93 ^{c,g,j,o}	8.84 ^{C}	9.58 ^{J}	8.04 ^{j,o}	8.92 ^{G}	8.99 ^{O}
	C2	8.10 ^{d,h,k,p}	9.85 ^{c,D}	10.01 ^{K}	8.12 ^{d,h,k,p}	9.21 ^{H}	9.38 ^{P}
	C3	8.02 ^{e,i,m,q}	10.44 ^{c,E}	9.72 ^{M}	8.27 ^{e,i,m,q}	9.60 ^{e,I}	9.19 ^{Q}
Conductivity (mS/cm)							
Anode	A1	0.79	1.05	0.89	0.77	0.76	0.79
	A2	0.71	0.95 ^{R}	0.76	0.60	0.90 ^{T}	0.71
	A3	0.75	0.99 ^{S}	0.81	0.54 ^{s}	0.93 ^{U}	0.75
Cathode	C1	0.41	0.65	0.50	0.79	0.62	0.41
	C2	0.40	0.44 ^{r}	0.44	0.54	0.37 ^{t}	0.40
	C3	0.33	0.46 ^{s}	0.38	0.55	0.40 ^{u}	0.33
Water content (%)							
Anode	A1	3.81	2.69 ^{x}	18.78 ^{e*}	2.63	2.33 ^{b*}	7.32 ^{i*}

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	A2	5.90 ^{v}	4.19 ^{V,y}	20.02 ^{f*}	5.28 ^{v}	3.99 ^{c*}	8.68 ^{j*}
	A3	7.09 ^{w,a*}	3.63 ^{W,z}	17.68 ^{g*}	7.73 ^{w,a*,h*}	6.28 ^{A*,d*}	12.20 ^{H*,k*}
Cathode	C1	3.89 ^{x,b*,e*,i*}	3.57 ^{X}	18.92 ^{E*}	3.19 ^{x,j*}	3.68 ^{B*}	7.84 ^{I*}
	C2	6.84 ^{y,c*,f*,j*}	5.90 ^{x,Y}	20.29 ^{F*}	6.28 ^{y,c*,f*,j*}	6.51 ^{C*}	9.14 ^{J*}
	C3	8.29 $^{z,d*,g*,k+}$	9.54 ^{x,Z}	17.96 ^{G*}	7.83 ^{z,d*,g*,k*}	9.69 ^{D*}	12.87 ^{z,K*}

∗ Experiments with current application (EK) were conducted with 20 mA.

Statistical analysis: Multiple comparisons were statistically performed at p<0.05 (95% confidence interval); data that has capital case letters is statistically significantly different with the accordingly lower letter).

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Table 2. Relative spatial distribution of the residual amount of each contaminant *per* soil section (% of the total mass *per* section) at the end of the 4-day experiments (round 1) is depicted schematically. Soil layer 3 (C3 and A3) not shown as no CECs were detected in these layers. (standard deviations and statistical analysis not shown for simplicity, detailed in ESI 7 and 8; n=2).

Experiment	Soil section	Biodegradable CECs				Recalcitrant CECs					
		E2	SMX	BPA	IBU	EE2	OXY	DCF	TCS	CAF	CBZ
Abiotic	A1	50	50	50	50	54	48	51	52	52	47
	A2	2	3	3	1	4	3	1	n.d.	2	4
	C1	43	46	43	47	40	44	43	48	45	45
	C2	4	3	5	3	3	5	4	n.d.	1	4
EK-Abiotic	A1	39	43	41	48	41	35	40	42	42	39
	A2	4	4	5	4	4	2	4	n.d.	n.d.	5
	C1	52	51	48	45	50	58	50	58	58	51
	C2	5	2	6	3	5	5	5	n.d.	n.d.	5
EK-Abiotic-W	A1	46	57	47	62	50	46	57	48	48	42
	A2	3	4	3	9	3	3	6	n.d.	n.d.	8
	C1	49	39	47	27	45	49	37	52	52	46
	C2	2	n.d.	3	2	2	2	1	n.d.	n.d.	4
Biotic	A1	46	47	52	47	49	47	51	48	49	50
	A2	5	3	2	1	3	5	3	n.d.	n.d.	5
	C1	49	42	46	52	48	48	45	52	51	45

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	C2	n.d.	9	n.d.	n.d.	n.d.	n.d.	1	n.d.	n.d.	n.d.
EK-Biotic	A1	38	42	44	38	43	44	43	48	46	42
	A2	n.d.	n.d.	4	0	2	2	n.d.	n.d.	n.d.	2
	C1	62	54	48	58	52	50	54	52	54	52
	C2	n.d.	4	3	4	3	4	3	n.d.	n.d.	5
EK-Biotic-W	A1	n.d.	37	41	56	38	42	46	46	50	41
	A2	n.d.	1	2	4	5	5	5	n.d.	n.d.	6
	C1	n.d.	60	56	40	56	50	48	54	50	50
	C2	n.d.	1	1	n.d.	1	3	1	n.d.	n.d.	3

Colours in the layer 1 represent CECs percentage: ≥60 orange; [30-40[green; ≤30 ciano.

n.d.: not detected

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Table 3. Soil pH, electric conductivity, water content by soil section for the round 2 experiments conducted for 4, 8 and 16 days. Mean soil temperature 19.0 ± 0.5 ºC (n=2). (standard deviations not shown for simplicity; available in ESI 9; n=2).

Soil		**Experiments round 2**					
		Biotic			**EK-Biotic**		
Section	**Layer**	**4d**	**8d**	**16d**	**4d**	**8d**	**16d**
pH							
Anode	A1	8.33 ^{d,i}	7.66 ^{d,l,m}	7.76 ^{d,l,m}	7.27 ^{a,i}	6.61 ^{D}	5.86 ^{l,o}
	A2	8.33 ^{e,j}	7.63 ^{e,j}	7.83 ^{e,j}	7.31 ^{b,j}	6.47 ^{E,j}	4.85 ^{J}
	A3	7.38 ^{k}	7.65 ^{f,k}	7.75 ^{f,k}	7.11 ^{c,k}	6.40 ^{F,k}	4.31 ^{l,K}
Cathode	C1	8.39 ^{a}	7.38	7.87	8.94 ^{A}	8.48 ^{h}	9.38 ^{l,M}
	C2	8.30 ^{n}	7.45 ^{b,g,n}	7.85 ^{b,g,n}	9.35 ^{B}	9.19 ^{e,G,n}	10.33 ^{j,N}
	C3	8.27 ^{c,h,o}	7.71 ^{c,h,o}	7.91 ^{c,h,o}	9.53 ^{C}	9.69 ^{f,H}	10.51 ^{k,O}
Conductivity (mS/cm)							
Anode	A1	0.74	0.63	0.58	0.84 ^{R}	0.59	0.48 ^{r}
	A2	0.60	0.58	0.52	0.79 ^{S}	0.51	0.50
	A3	0.64	0.61	0.61	0.88 ^{T}	0.69	0.56 ^{t}
Cathode	C1	0.73 ^{P}	0.62 ^{Q}	0.59	0.38 ^{p,r}	0.32 ^{p}	0.30 ^{p,q}
	C2	0.61	0.54	0.56	0.36 ^{s}	0.32	0.34
	C3	0.62	0.60	0.57	0.42 ^{t}	0.39	0.38
Water content (%)							

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Anode	A1	9.8 ^{y}	10.0 ^{y}	12.2 ^{y}	5.5 ^{Y}	9.9 ^{y}	11.3 ^{y}
	A2	10.2 ^{z}	10.9 ^{z}	12.2 ^{z}	6.3 ^{Z}	10.8 ^{z}	11.5 ^{z}
	A3	10.7	10.0	11.6 ^{X}	9.0 ^{x,z}	10.3	11.0
Cathode	C1	9.2	8.7	11.2	9.0 ^{y}	10.8	10.3
	C2	10.2	11.0	12.0 ^{W}	8.9 ^{w,z}	11.5	11.1
	C3	9.8 ^{V}	10.9	13.3 ^{v}	10.9	12.0	12.3

∗ Experiments with current application (EK) were conducted with 20 mA.

Statistical analysis: Multiple comparisons were statistically performed at p<0.05 (95% confidence interval); data that has capital case letters is statistically significantly different with the accordingly lower letter).

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Table 4. Estimated kinetic parameters of the CECs decay for the Biotic experiments conducted on round 2 (graphical representation available in ESI 12).

CEC	Type	Pseudo-first order			Pseudo-first order kinetic with lag phase				Polynomial		
		k (h^{-1})	$t_{½}$ (days)	R^2	Lag phase (h)	k (h^{-1})	$t_{½}$ (days)	R^2	K_1	K_2	R^2
E2 [(a)]	I	3.17×10^{-2}	0.9	1.000	-	-	-	-	-	-	-
SMX	I	5.10×10^{-3}	5.7	0.980	-	-	-	-	-	-	-
BPA	I	6.40×10^{-3}	4.5	0.998	-	-	-	-	-	-	-
IBU	II	-	-	-	96	6.4×10^{-3}	8.5	0.951	1.9×10^{-3}	-5.0×10^{-6}	0.998
EE2	II	-	-	-	96	4.5×10^{-3}	10.4	0.941	9.0×10^{-4}	-4.0×10^{-6}	0.998
OXY	III	-	-	-	192	2.3×10^{-3}	20.6	0.906	2.0×10^{-4}	-4.0×10^{-6}	0.999
DCF	II	-	-	-	192	6.0×10^{-3}	12.8	0.856	1.2×10^{-3}	-1.0×10^{-5}	1.000
TCS	III	-	-	-	192	1.4×10^{-3}	28.6	0.882	2.0×10^{-4}	-2.0×10^{-6}	1.000
CAF	II	-	-	-	192	6.9×10^{-3}	12.2	0.830	1.6×10^{-3}	-1.0×10^{-5}	0.998
CBZ	III	-	-	-	192	1.3×10^{-3}	30.2	0.868	2.0×10^{-4}	-2.0×10^{-6}	1.000

[a] *E2 data is only presented for comparison reasons as it achieved 100% decay on day 8, thus data was only available for day 0 and 4.*

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Table 5. Relative spatial distribution of the residual amount of each contaminant per soil section (% of the total mass per section) at the end of the round 2 experiments is depicted schematically. Soil layer 3 (C3 and A3) not shown as no CECs were detected in these layers. (Standard deviations and statistical analysis not show for simplicity, detailed in ESI 13 and 14; n=2).

Exp.	Day	Soil section	CEC									
			E2	SMX	BPA	IBU	EE2	OXY	DCF	TCS	CAF	CBZ
Biotic	4	A1	60	55	56	60	58	56	55	55	48	43
		A2	n.d.	n.d.	2	n.d.	2	2	1	n.d.	n.d.	n.d.
		C1	40	43	41	40	40	41	42	45	52	55
		C2	n.d.	2	1	n.d.	1	1	2	n.d.	n.d.	3
	8	A1	n.d.	61	53	61	51	51	53	55	54	52
		A2	n.d.	1	3	n.d.	3	3	3	n.d.	n.d.	3
		C1	n.d.	38	43	39	42	42	40	45	46	40
		C2	n.d.	9	1	n.d.	4	4	4	n.d.	n.d.	5
	16	A1	n.d.	60	50	60	49	49	51	47	50	48
		A2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		C1	n.d.	40	50	40	51	51	49	53	50	48
		C2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	4
EK-Biotic	4	A1	44	35	40	35	40	40	41	44	48	44
		A2	n.d.	4	3	7	2	4	3	n.d.	n.d.	5
		C1	50	53	54	53	53	52	53	56	52	48

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		C2	6	8	3	5	4	4	3	n.d.	n.d.	4
	8	A1	n.d.	100	28	96	34	35	74	37	40	40
		A2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		C1	n.d.	n.d.	72	4	66	61	26	63	60	55
		C2	n.d.	n.d.	n.d.	n.d.	n.d.	3	n.d.	n.d.	n.d.	5
	16	A1	n.d.	100	42	100	44	39	87	47	44	39
		A2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1
		C1	n.d.	n.d.	58	n.d.	56	56	13	53	56	55
		C2	n.d.	n.d.	n.d.	n.d.	n.d.	5	n.d.	n.d.	n.d.	6

Colours in the layer 1 represent CEC percentage: ≥80 orange; [60-80[yellow; [30-40[green; ≤30 ciano

n.d.: not detected

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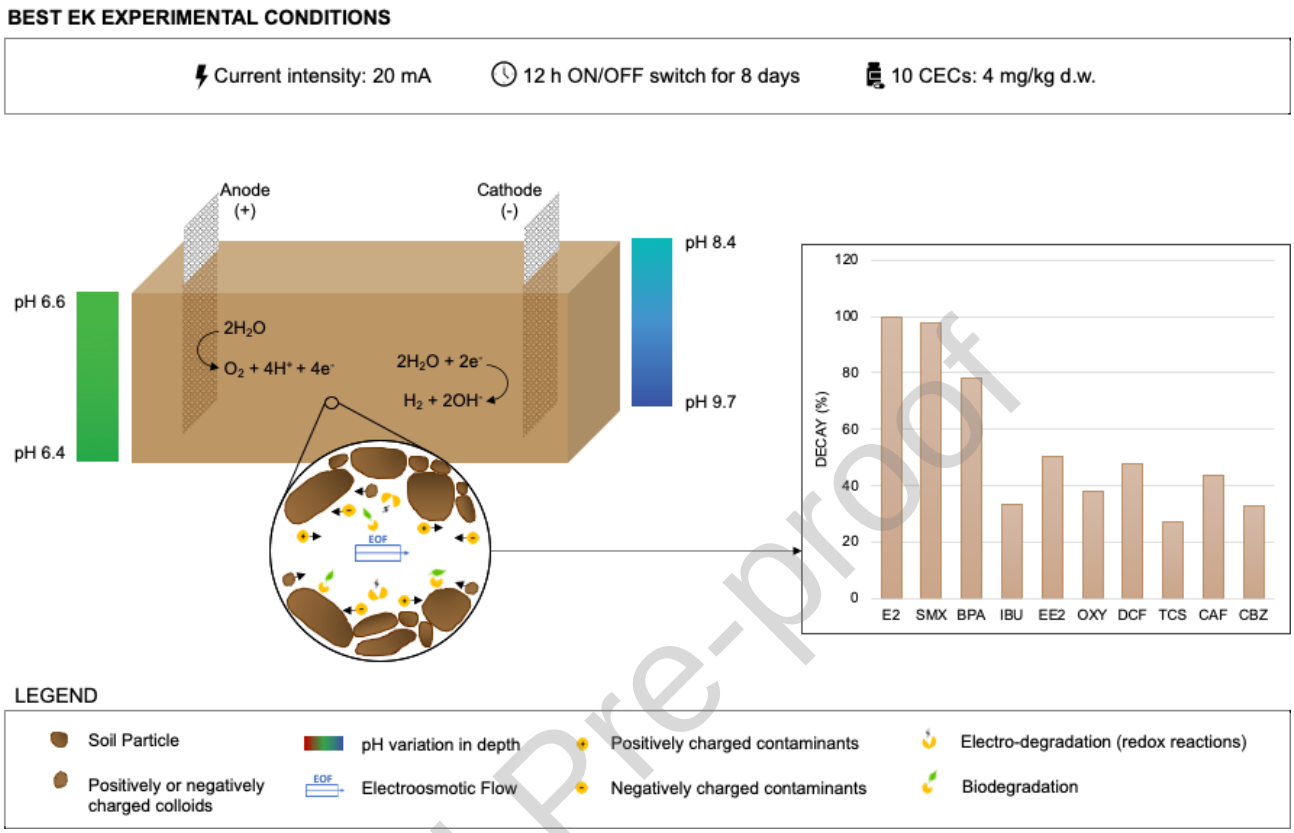
Graphical abstract



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Author contributions

P. Guedes: Conceptualization, Methodology, Investigation, Writing - Original Draft; **J. Dionísio**: Investigation, Formal analysis, Writing - Original Draft; **N. Couto**: Methodology, Resources, Writing - Review & Editing, Funding acquisition; **E.P. Mateus**: Validation, Resources, Writing - Review & Editing, Methodology; **C.S. Pereira**: Supervision, Methodology, Writing - Review & Editing, Funding acquisition; **A.B. Ribeiro**: Supervision, Writing - Review & Editing, Resources, Methodology, Funding acquisition.

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Declaration of interests

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

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

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Highlights:

- EK-assisted remediation accelerated the decay of most CECs in a clay soil.
- CECs recalcitrant to biodegradation mostly underwent electro-chemical degradation.
- Irrigation increased the CECs decay levels.
- Applying EK for 16 days altered the soil pH and decreased removal efficiencies.
- EK conditions should target the recalcitrant CECs while ensuring bioremediation efficiencies.

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