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Electrokinetic remediation of PPCPs in soil: influence of soil biota and environmental factors

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RESUMO

A utilização de águas residuais tratadas para irrigação agrícola é uma prática comum em vários países, diminuindo assim a pressão sobre as fontes de água doce. No entanto, as estações de tratamento de águas residuais (ETAR) nem sempre são capazes de remover completamente os contaminantes presentes na água, representando um risco de contaminação ambiental. A contaminação do solo por águas residuais pode potencialmente promover a absorção e a acumulação de contaminantes por plantas e produtos hortícolas que, consequentemente, podem afetar a saúde humana. Os agentes patogénicos e os metais pesados são tradicionalmente a principal preocupação, no entanto os contaminantes de preocupação emergente (CECs), e particularmente os produtos farmacêuticos e de cuidado pessoal (PPCPs), têm vindo a captar a atenção científica e pública.

Na presente tese, o processo eletrocinético (EK) foi aplicado como tecnologia de remediação a um solo agrícola contaminado com uma mistura de PPCPs. Para melhor compreender os mecanismos de remediação envolvidos, foram desenvolvidos diferentes ensaios para discriminar contribuições bióticas (solo não esterilizado), abióticas (solo esterilizado) e do processo EK, isoladas e acopladas, para a degradação dos PPCPs. Como os fatores ambientais podem afetar a remediação, foram realizados ensaios com e sem irrigação e a diferentes temperaturas (18 e 24ºC). Como contaminantes foram usados 10 PPCPs: 17β-estradiol (E2), sulfametoxazol (SMX), bisfenol A (BPA), ibuprofeno (IBU), 17α-etinilestradiol (EE2), oxibenzona (OXY), triclosan (TCS), diclofenaco (DCF), cafeína (CAF) e carbamazepina (CBZ). Estes compostos são representativos das principais classes de PPCPs e abrangem diversas propriedades físico-químicas. Todas as experiências foram realizadas em microcosmos à escala laboratorial usando 20 mA em modo de corrente ON/OFF de ciclos de 12h por 4 dias (em duplicado).

Os melhores resultados de remediação foram obtidos quando o processo EK foi aplicado em condições bióticas a 24ºC, em combinação com irrigação diária; estas condições permitiram remover aproximadamente 37% da massa total de PPCPs no solo. Ao eliminar-se a contribuição microbiológica para os processos de degradação (solo esterilizado; ambiente abiótico) nas mesmas condições de EK, promoveu-se uma diminuição da remoção em 7%. Os mecanismos de remoção abiótica contribuem apenas para um declínio de massa total próximo dos 6% enquanto que o mecanismo biótico permite uma remoção de 20% (a 24ºC). Os PPCPs mais recalcitrantes à degradação foram a CBZ, a OXY e o TCS. Em oposição o E2, SMX e BPA demonstraram ser mais biodegradáveis.

Os resultados obtidos corroboram que o uso combinado de EK com a biorremediação melhora consideravelmente a eficiência da remoção de poluentes do solo. O processo EK é uma opção eficaz para a remediação de PPCPs em solos argilosos, potencializando a degradação de contaminantes através de dois principais mecanismos de remediação: (i) degradação eletroquímica induzida e (ii) biorremediação.

Palavras-chave: Processo eletrocinético; biorremediação; remoção abiótica; solo agrícola; contaminantes de preocupação emergente

ABSTRACT

The use of treated wastewater for agricultural irrigation, is a common practice in several countries as it has several benefits, such as decreasing pressure on freshwater sources and reduced nutrient loads to receiving waters. However, wastewater treatment plants (WWTP) are not always able to remove all the contaminants present, thus representing a significant risk for environmental contamination. Soil contamination by wastewater may potentially promote contaminants uptake and accumulation by plants and derived products which consequently can affect human health. Pathogens and heavy metals are traditionally the main concern, however contaminants of emerging concern (CECs), and particularly pharmaceutical and personal care products (PPCPs), are gaining scientific and public attention.

In the present dissertation, the electrokinetic (EK) process was applied as a remediation technology to an agricultural soil contaminated with a mixture of PPCPs. To better understand the remediation mechanisms involved, different assays were developed to discriminate biotic, abiotic and EK contributions, alone and coupled, to the degradation of the PPCPs. As environmental factors may affect the remediation processes, trials with and without irrigation and at different temperatures were also conducted (18 and 24 ºC). As contaminants, 10 commonly environmentally occurring PPCPs were selected for the study: 17β-estradiol (E2), sulfamethoxazole (SMX), bisphenol A (BPA), ibuprofen (IBU), 17α-ethinylestradiol (EE2), oxybenzone (OXY), triclosan (TCS), diclofenac (DCF), caffeine (CAF) and carbamazepine (CBZ). These compounds represent the major PPCPs classes and attain diverse physicochemical properties. All experiments were carried out in a lab scale microcosm using a 12h ON/OFF current mode at 20 mA for 4 days (in duplicates).

The best remediation results were attained when EK process was applied in biotic conditions at 24ºC and combined with a daily irrigation (EK-Biotic-24-W); these conditions allowed to remove approximately 37% of the total mass on PPCPs in the soil. By eliminating the microbiological contribution to the degradation processes (sterilized soil; abiotic setting) in the same EK conditions (EK-Abiotic-24-W) a decrease in the removal by 7% was observed. The abiotic removal mechanisms only contributed to a total mass decay of 6%, whereas the biotic mechanism removed 20% (at 24ºC). The PPCPs more recalcitrant to degradation were CBZ, OXY and TCS. Oppositely E2, SMX e BPA displayed to be more biodegradable.

Such results are compatible with previous tests, which suggest that usage of EK in combination with bioremediation vastly improves the efficiency over that of EK remediation alone in removing pollutants from soil. The EK process showed to be an effective option for the remediation of PPCPs in clay soils, which may enhance the degradation of contaminants by two main remediation mechanisms: (i) electrochemical induced degradation and (ii) bioremediation.

Keywords: Electrokinetic process; bioremediation; abiotic removal; agricultural soil; contaminants of emergent concern

INDEX

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS AND SYMBOLS

ACN	acetonitrile
BPA	bisphenol A
CAF	caffeine
CBZ	carbamazepine
CEC	cation exchange capacity
CECs	compounds of emerging concern
CI	current intensity
DAD	diode array detector
DC	direct current
DCF	diclofenac sodium salt
EC	electric conductivity
EDCs	endocrine disrupting compounds
E2	17β-estradiol
EE2	17α-ethinylestradiol
EK	electrokinetic
EOF	electroosmotic flux
EU	European Union
FLD	fluorescence detector
HPLC	high-performance liquid chromatography
IBU	ibuprofen
LD	limit of detection
LQ	limit of quantification
MLD	method limit of detection
MLQ	method limit of quantification
OXY	oxybenzone
PPCPs	pharmaceuticals and personal care products
QuEChERS	quick easy cheap effective rugged and safe
SMX	sulfamethoxazole
SPE	solid-phase extraction
TCS	triclosan
UV	ultraviolet
WWTP	wastewater treatment plant

1. Introduction

Sustainable water management has become an issue of major importance over the past decades. One fundamental concern is the impact of climate change and increasing population on water supply, which will add to the problems of water scarcity and droughts. While Europe is considered as having adequate water resources, water scarcity is an increasingly frequent and widespread phenomenon in the European Union (EU), where approximately half of the European countries, representing almost 70% of the population, are facing water stress issues (Bixio *et al*, 2006). A third of water use in Europe goes to the agricultural sector and agriculture accounts for 70% of global freshwater withdrawals (FAO, 2017; EEA, 2012). In long term this affects both the quantity and the quality of water available for other uses. More specifically, in Portugal, agriculture and livestock are the dominant sector, accounting for approximately 75% of water use, followed by supply to populations with 20% and industry with 5% (APA, 2016).

To address these demands and others emerging ones, water resources management approaches around the world are changing and shifting away from sole reliance on finding new sources of supply and gaining emphasis on incorporating ecological values into water policy (Gleick *et al*., 2000). As a response, municipal wastewater reuse has been developed as an important and viable mean of supplementing decreasing water supplies and promoted as a way of limiting wastewater discharges to aquatic environments (Weber *et al*., 2006).

Although wastewater seem to be a good alternative, one of the challenges is the establishment of guidelines or criteria. World Health Organization (WHO) guiding principles must therefore be practical and offer viable risk-management solutions that minimize health risks and allow for the beneficial use of scarce resources. Recently this year (February), the European Parliament has adopted rules to facilitate the reuse of water in the EU for agricultural irrigation and to help manage water scarcity and droughts. The European Parliament and the Council on Water Scarcity and Droughts sets out the hierarchy of measures that Member States should consider in managing water scarcity and droughts, accenting that saving water must become the priority and all strategies to improve water efficiency should be explored. Setting minimum requirements for all member states on the quality of reclaimed water and monitoring, together with responsible risk management tasks ensures equity for those engaged in water reuse and those affected, preventing potential obstacles to the commercialization and consumption of agricultural products irrigated with reclaimed water. It also allows to ensure health and environmental protection which increases confidence in the practice of water reuse.

The uncontrolled use of wastewater in agriculture may have significant health consequences for consumers, farmers and communities in wastewater-irrigated areas, since it can damage the soil and the quality of the crops affected by the different types of pollutants. This could lead to a deteriorating quality of groundwater and soil (Erikson *et al*., 2006; Carr *et al*., 2004). According to Candela *et al.,* (2007), treated wastewater may still contain humic substances, heavy metals, pesticides, disinfection by-products, industrial contaminants, microorganisms, inorganic and organic compounds, including Pharmaceuticals and Personal

Care Products (PPCPs) - many of them with unknown geochemical behaviour, raising concerns regarding their potential environmental fates and effects on human health.

Consistent with Ternes et *al*., (2004), in the EU there are more than 3000 different substances used as medicines, including painkillers, antibiotics, contraceptives, beta-blockers, lipid regulators, tranquilizers, and impotence drugs. During and after treatment, humans and animals excrete a combination of intact and metabolized pharmaceuticals, many of which are generally soluble in water and have been discharged to the environment with little evaluation of possible risks or consequences to humans and the environment. The environmental toxicology of PPCPs is not well understood, and toxicological concerns regarding the environmental release of these compounds include inducement of abnormal physiological processes and reproductive impairment, increased incidences of cancer, development of antibiotic resistant bacteria, and the potential for increased toxicities when chemical mixtures occur in the environment (Richardson *et al*., 2005).

PPCPs enter the soil environment via irrigation with treated wastewater, groundwater recharge, and land application of biosolids. The transformation and fate of PPCP in soil affects their potential for plant uptake and groundwater pollution (Dodgen *et al*., 2014), since significant amounts of PPCP compounds can be found accumulated in the top soil profile at an irrigated field (Xu *et al*., 2009; Chen *et al*., 2013). Irrigation with treated wastewater may cause accumulation of PPCPs in soil to higher levels (than in the irrigation water) and soil half-lives for PPCP can vary widely depending on the compound (ranging from hours in the case of ibuprofen, to years in the case of fluoxetine) and environmental conditions (Dodgen, 2014).

The electrokinetic (EK) soil remediation process was proposed as an effective *in situ* and *ex situ* technology to remove heavy metals and organic contaminants from contaminated soil (Wang *et al.*, 2007), by directing contaminant migration to where remediation may be more easily achieved (Lear *et al.*, 2007). This technique has already demonstrated its effectiveness in soils contaminated with PPCPs (Guedes *et al*., 2014; Ferreira *et al*., 2017; Li *et al*., 2018; Silva, 2018; Lopes, 2018).

This procedure relies on the application of a low-density direct current between electrodes placed in the soil (Kim *et al.,* 2000). The electric field applied across a saturated soil mass results in electrolysis reactions, transport of species by ionic migration, electroosmosis, and diffusion. These transport processes are accompanied by sorption processes in the soil, precipitation and dissolution, and other aqueous phase reactions in the pore fluid (Acar *et al*., 1995).

1.1. Study objectives and research

The main objective of this dissertation was to study the EK remediation of soils contaminated with PPCPs, aiming to decrease the risk of organic contaminants uptake by crops. The present dissertation proposes to answer the following questions:

a) Is the EK process a viable *in situ* technology for the remediation of a mixture of 10 organic contaminants?

b) How does the different removal mechanism (abiotic, biotic, electrokinetic processes) contribute to the PPCPs removal?
c) How do the environmental parameters, like water content and temperature, affect the PPCPs degradation rates?
d) How does an organic contaminant mobilize in the soil with and without EK?

To answer these questions, a microcosm capable of simulating *in situ* conditions was designed, and experiments were performed at a laboratory scale. The assays (duplicates) were carried out using an agricultural clay soil used for organic tomato field plantation. All experiments were carried out in a lab scale microcosm for 4 days using as EK operating parameter a 12h ON/OFF current mode at 20 mA (previously optimised by Lopes, 2018).

In total eight settings were tested in duplicate, six at 24°C and two at 18°C and. At 24°C it was possible to study the effect of different parameters inside flow chamber that promote sterile conditions: Biotic *vs* Abiotic - which disclaimed the contribution of biodegradation; EK-Biotic-24 vs EK-Abiotic-24 - allowed to analyse the EK efficiency alone and coupled to bioremediation; and EK-Biotic-24-W and EK-Abiotic-24-W - allowed to assess the impact of soil moisture (daily irrigation). At 18°C two different scenarios were tested: EK-Biotic-18 and Biotic-18 - disclaimed the contribution of lower temperature to the PPCPs removal. A time zero control was also performed.

In its entirety, ten compounds belonging to the group of pharmaceuticals and personal care products, were used to spike the soil, those were: 17β-estradiol (E2), sulfamethoxazole (SMX), bisphenol A (BPA), ibuprofen (IBU), 17α-ethinylestradiol (EE2), oxybenzone (OXY), triclosan (TCS), diclofenac (DCF), caffeine (CAF) and carbamazepine (CBZ). These compounds were chosen since they are commonly occurring on WWTP effluents and belong to different classes also comprising different physicochemical properties.

To accomplish this work, all laboratorial work was carried out at the RESOLUTION Lab (347; CENSE) and at the “Soil teaching Laboratory” (231; DCEA, FCT NOVA).

1.2. Dissertation structure

The present dissertation is organized in the following chapters:

I. Introduction – work scope and relevance, main objectives and structure;
II. Literature review – description of the central theme and relevant terms and previous work developed;
III. Materials and methods – description of materials used, characterization analysis, identification and data treatment methods;
IV. Results and discussion – presentation of results, hypothesis formulation and their discussion;
V. Conclusions – main outcomes;

VI. Future developments;

VII. References;

VIII. Annexes – includes a set of detailed data complementary to the main document

2. Literature review

2.1 Wastewater reuse

Global water shortage is placing an unprecedented pressure on water supplies, and so creating a need to find more ecological alternatives. With such scenario treated wastewater becomes a valuable resource. Irrigation with reclaimed wastewater is one of the most important applications as roughly 2/3 of all water use goes to agriculture irrigation (Baresel, *et al*., 2015).

The use of treated wastewater in agriculture benefits human health, the environment and the economy. These benefits include increased water availability by decreasing pressure on freshwater sources; reduced over-abstraction of surface and groundwater and, consequently, reduced energy consumption compared to using deep groundwater resources and reduced nutrient loads to receiving waters (Sanz & Gawlik, 2017). Also, there is a rapidly growing world water market, which is estimated to be as large as 1 trillion Euros by 2020. By seizing new and significant market opportunities, Europe can increasingly become a global market leader in water-related innovation and technology (BIO by Deloitte, 2015).

The level of treatment before discharge and the sensitivity of the receiving waters determine the scale of the impacts on aquatic ecosystems. In the WWTPs, primary (mechanical) treatment removes some of the suspended solids, while secondary (biological) treatment uses aerobic or anaerobic microorganisms to decompose most of the organic matter and retain some of the nutrients (around 20-30%). Tertiary (advanced) treatment removes organic matter even more efficiently (85%). It generally includes phosphorus retention and, in some cases, nitrogen removal (Kosma *et al.,* 2014; Lubliner *et al.,* 2010). In Figure 2.1 is possible to observe the evolution of type of water treatment applied in Portugal since 1980 until 2015.

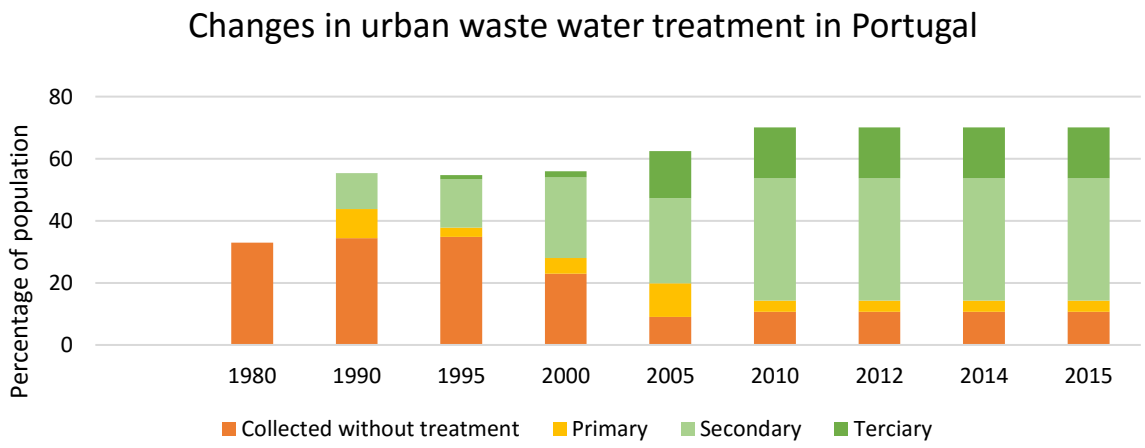


Figure 2.1 Type of water treatment applied in Portugal since 1980 until 2015, related to the percentage of population served by each type of treatment (Source: EEA, 2012).

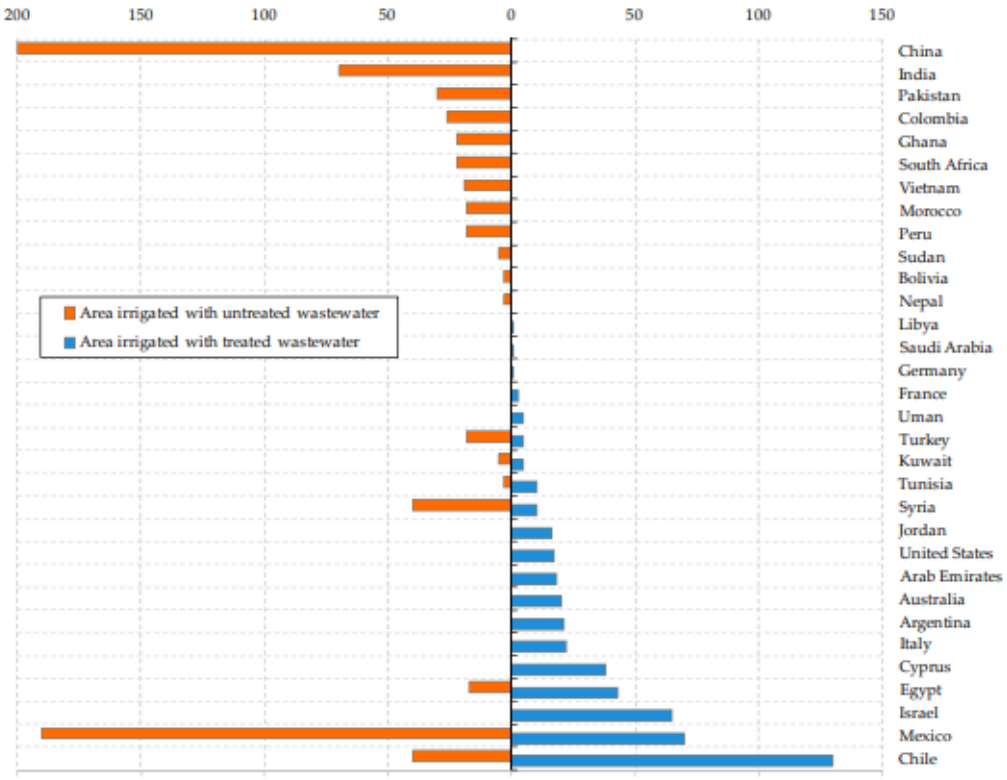


Figure 2.2 Reuse area in agriculture by country (thousand ha) (Jaramillo & Restrepo, 2017).

FAO reported that approximately 10% of the total global irrigated land area receives untreated or partially treated wastewater, resulting in 20 million hectares in 50 countries (Figure 2.2).

Standards adopted by Member States for water reuse specify intended uses which are mostly agricultural and urban applications. The Spanish and the Greek standards are those for which the highest number of uses is included. In comparison the Portuguese guidelines only refer the use for irrigation of urban areas and agriculture (European Commission (2018)). While water reuse is certainly a promising option for many Member States, it needs to be considered that at present only 6 Member States (Cyprus, Greece, Spain, France, Italy and Portugal) have requirements on water reuse in place (in legislation or in national non-regulatory standards) (European Commission (2018).

The use of treated or untreated wastewater in agriculture is not exempt from adverse effects on the environment, especially on soil (Table 2.1). The scientific literature includes evidence of alterations in the physicochemical parameters of soil. Additionally, in recent research, variations have been observed in the structure and magnitude of microbial biomass in soil, as well as an increase in microbial activity caused by agricultural wastewater reuse (Jaramillo *et al.*, 2017). Altering physicochemical parameters and soil microbiota can affect fertility and

productivity, thus disturbing soil sustainability from inadequate irrigation with wastewater (Becerra *et al*., 2015).

Table 2.1 Effects on the environment from the use of wastewater in agriculture (Jaramillo et al., 2017)

Parameter	Associated Effects on the Soil and the Environment	
	Physicochemical Properties	**Microbiological Properties**
pH	Increases the availability of nutrients and trace metals Mineralization of organic matter Cation exchange capacity	Community richness and diversity
Organic matter	Soil structure stabilization Formation of aggregates Water retention Improves nutrient content Buffer Capacity Cation exchange capacity Enzymatic activity Increase in TOC Possible increase of contaminants availability	Selection of specific populations Soil microhabitats
Nutrients	Increase in organic soil matter Water retention Leaching to groundwater Improves nutrient content Risk of eutrophication of aquatic environments	Perturbation of microbial soil communities Microbial catabolic activity
Salinity	Soil salinization or sodification Decreased stability of aggregates Changes in soil structure in the long term Permeability of soil and water retention Increased soil compaction Variation in soil pH Negative impact on soil fertility Dynamics in organic and inorganic compounds Heavy metal leaching	Changes in soil microhabitats and variation in diversity and activity of the microbial community
Contaminants	Soil toxicity and leaching Accumulation in soils Negative impact on soil fertility Potential contamination of the food chain Mineralization of organic matter Changes in enzyme activity Decomposition of fallen leaves Limiting soil fertility	Community structure and diversity Increase of microbial tolerance to contaminants and/or biodegradation Spread of antibiotic resistance

The hygienic quality of the wastewater is the major aspect to consider when wastewater is used in agriculture. One challenge of promoting the use of treated wastewater on agricultural

irrigation is the safety concern of its products due to the presence of various pollutants in treated wastewater (Wu *et al.,* 2014).

The concentration levels and types of pathogens and chemical substances present in wastewater vary by region, according to the sanitary and socioeconomic conditions of a community. Besides viruses, protozoan parasites and helminths that can be found in irrigated wastewater, other compounds that may pose risks to human health are emerging contaminants, particularly PPCPs (Jaramillo *et al.,* 2017) which rightfully have become a new issue garnering public attention (Wang *et al*., 2005)

Due to the increasing human use of PPCPs with aging populations and advances in healthcare, and the fact that WWTP are generally incapable of completely removing these chemicals, PPCPs are universally found in WWTP effluent around the world, with levels ranging from µg/L to low ng/L (Vanderford *et al*., 2006; Wu *et al*., 2014; Gros *et al*., 2010). In 2010, the Washington State Department of Ecology (Ecology) and the U.S. Environmental Protection Agency (EPA) conducted a one-day screening study to characterize PPCPs at five municipals WWTPs in the Pacific Northwest. Secondary treatment alone achieved high removals for hormones and steroids. Approximately 21% of the 172 analytes were reduced to below reporting limits by conventional secondary treatment, whereas 53% were reduced to below reporting limits by at least one advanced nutrient-removal technology. Roughly 20% of the 172 analytes (mainly polycyclic aromatic hydrocarbons) were found only in the biosolids and not in the wastewater samples. Some analytes were clearly concentrating in the biosolids. Three PPCPs (carbamazepine, fluoxetine, and thiabendazole) were relatively untreated by the surveyed WWTP technologies (Lubliner *et al.,* 2010).

These PPCPs are molecules with biological activity on different organisms, and their physicochemical properties determine their persistence in the environment and facilitate their bioaccumulation. The effluents of municipal wastewater treatment plants are classified as one of the primary PPCPs sources, as conventional treatment processes do not effectively prevent the release of these compounds into the environment (Jaramillo *et al.*, 2017; Jackson *et al.,* 2008). Albeit at trace levels in the effluents, PPCPs will probably accumulate in the soils if long-term irrigation occurs, which may result in environmental problems such as the contamination risk to groundwater (Baresel *et al.,* 2005).

2.2 PPCPs

Compounds of emerging concern (CEC) are a chemical group of contaminants of which PPCPs and endocrine disrupting chemicals (EDCs) are included. These compounds produce biological activity on different organisms, and their physicochemical properties determine their persistence in the environment and facilitate their bioaccumulation. As said before, effluents of municipal WWTPs are classified as one of the primary PPCPs sources, as conventional treatment

processes do not effectively prevent the release of these compounds into the environment (Jaramillo *et al.*, 2017; Jackson *et al*., 2008).

PPCPs form a diverse group of chemicals comprising human and veterinary drugs, diagnostic agents such as X-ray contrast media, bioactive food supplements and other consumer chemicals such as fragrances, cosmetics and sun-screen agents as well as inert ingredients or excipients used in PPCP formulations and manufacture. Table 2.2 lists some major PPCP functional classes in terms of their use and the associated principal compounds of environmental concern (Ellis, 2008). Their adsorption behaviours vary from compound to compound and are difficult to predict because their behaviour is frequently controlled by interactions with specific functional groups or complicated pH-dependent speciation (Kibbey *et al.*, 2007).

Table 2.2 PPCPs organized by functional classes in terms of their main use.

COMPOUND GROUP/CLASS	COMPOUND
Pharmaceuticals	
Veterinary & Human Antibiotics.	Trimethoprim (710), Erytromycine, Lincomycin, Sulfamethaxole (130), Chloramphenicol (355), ***Amoxycillin***, ***Flucloxacillin***.
Analgesics & Anti-inflammatory Drugs.	***Ibuprofen*** (3400), Diclofenac 1200), Fenoprofen, ***Acetaminophen*** (10,000), Naproxen (390), ***Acetylsalicylic acid*** (340), Fluoxetine (12), Ketoprofen (120), Indometacine (200), ***Mesalazine***, ***Sufasalazine***.
Psychiatric Drugs.	Diazepam, ***Carbamazepine*** (1100), Primidone, Salbutamol (35).
Lipid Regulators.	Clofibric acid (40), Bezafibrate (3100), Fenofibric acid, Etofibrate, Gemfibrocil (790), ***Metformin*** (150).
β-Blockers.	Metoprolol (2290), Propranolol (590), Timolol (10), Sotalol, Atenolol.
X-ray Contrasts.	Iopromide, Iopamidol, Diatrizoate (100,000).
Steroids & Hormones.	Estradiol, Estrone, Estriol, DES.
Personal Care Products	
Fragrances	Nitro, polycyclic and macrocyclic Musks; Phthalates
Sun Screen Agents	Benzophenone, methylbenzylidene camphor.
Insect Repellents	N,N-diethyloluamide.
Antiseptics	Triclosan, Chlorophene.

Another recent aspect of concern about PPCPs is the overuse and misuse of antibiotics which may result in the release of resistance vectors into the environment. The rise of antibiotic resistance is considered to possibly be related with the widespread use of antibiotic pharmaceuticals in humans and animals. In both humans and animals, up to 95% of antibiotics can be excreted in an unaltered state (Pruden *et al.,* 2006). Without adequate treatment at the WWTP residual antibiotics are consequently released into the environment where they may exercise selection pressure on microorganisms.

i. Impacts in environment and food chain

Municipal wastewater, attributed to the widespread use of PPCPs both in the home and in health care and personal care facilities, is the primary pathway by which chemicals in

prescription and over-the-counter products find their way into the aquatic environment (Wenning *et al.,* 2014).

Other pathways for PPCPs to enter the environment are: aquaculture facilities, and runoff from fields, as well as releases to soils during biosolid and manure application, emissions from manufacturing sites, disposal of unused medicines to landfills, runoff of veterinary medicines in farmyards, off label emissions, and disposal of carcasses of treated animals (Boxall *et al.,* 2012). A schematic of the several possible entryways of these compounds is depicted in Figure 2.3.

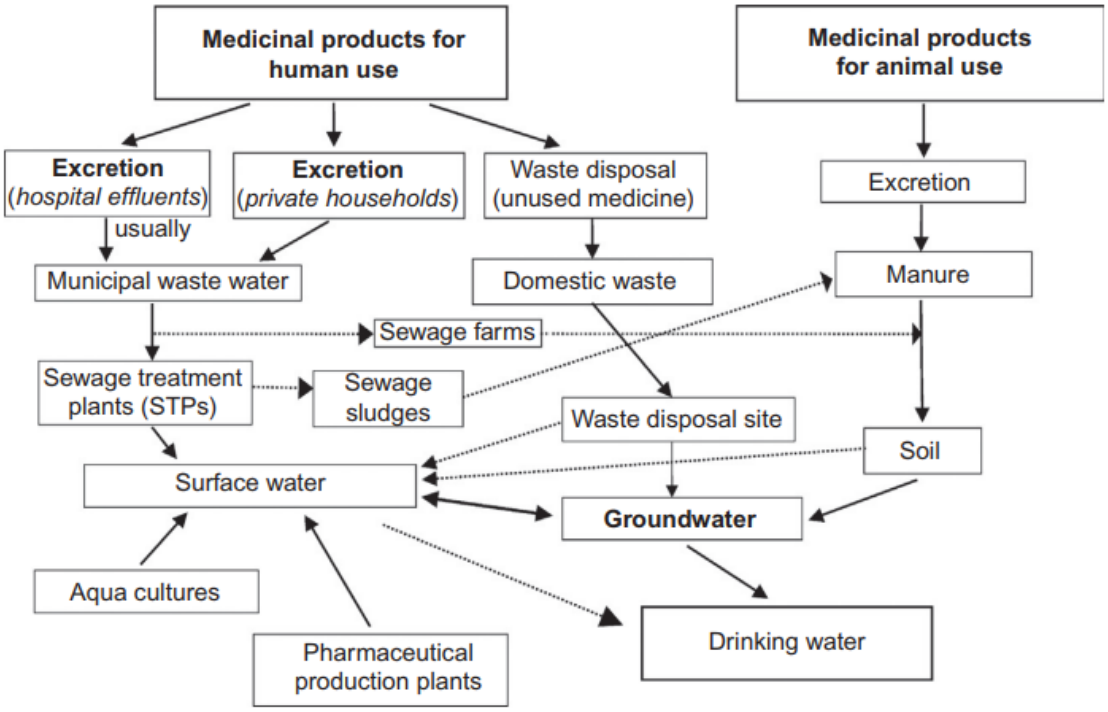


Figure 2.3 Possible sources and pathways for the occurrence of pharmaceutical residues in the aquatic environment (Source: Heberer, et al., 2002).

When treated wastewater is used for agricultural irrigation, contaminants in reclaimed water may transfer to crops from soil through root uptake and translocation, and the risk is higher for products that may be consumed raw (e.g. fresh fruits, vegetables). (Wu *et al.,* 2014; Kosma *et al*., 2014; Grassi *et al*., 2013; Miege *et al*., 2019).

Antibiotics are usually the most-abundant PPCPs in plants because of the high concentration in the biosolids and animal manure applied to agricultural fields. The physicochemical properties of the compounds like hydrophobicity and ionization, have great influence in the uptake, accumulation, translocation, and transformation of PPCPs in plants. Soil properties, such as water and organic matter content, pH, the duration and concentration of the exposure, and lastly the physiological nature of the plant and its tissues can also affect the uptake and accumulation of PPCPs (Bartrons & Peñuelas, 2017).

A study by Wu *et al.,* (2014) showed that when irrigated with treated wastewater, common vegetables grown under field conditions were capable of selectively accumulate PPCPs into their edible parts, and at different frequencies and levels. Among the 8 vegetables studied by these researchers the cumulative *per* capita annual exposure to PPCPs was found to be the highest in carrot (2.33 µg), followed by lettuce (0.53 µg), celery (0.31 µg), cabbage (0.25 µg), and bell pepper (0.14 µg), while exposure values for spinach, cucumber and tomato were small (<0.09 µg). The contaminant that through consumption of contaminated vegetables was accountable for greatest annual exposure was caffeine (1.25 µg), followed by triclosan (0.84 µg), and carbamazepine (0.64 µg). The ionic state of the compound greatly affects the compound's interactions with plants, such as adsorption on root surfaces, interaction with the cell membrane, and sequestration into plant compartments (Trapp, 2009).

Consumption of these PPCP-contaminated vegetables represents an exposure pathway for humans via dietary intake. However, most studies about the risks of PPCPs on both environmental end-points and human receptor targets indicate a low risk of adverse effects in the various trophic levels of food webs or in human health. This conclusion is based on the low concentrations of PPCPs in plants and the low toxicity of most compounds (exposure levels are usually below human therapeutic dose). However, there is an increasing concern since there is increasing selection of antibiotic-resistant microorganisms in the environment, including pathogens, and because it may occur possible synergistic effects between PPCPs and other micropollutants or medications taken by a patient for an existing condition (Bartrons & Peñuelas, 2017).

Actually, some PPCPs can have dramatic adverse effects on wildlife. For example, the inappropriate use of diclofenac and disposal of animal carcasses, combined with the high sensitivity of vultures to diclofenac, were responsible for the decline in populations of three vulture species in Asia (Oaks *et al.,* 2004). The oestrogens E2 and EE2 are also associated with severe health effects in some species of fish, impacting the sustainability of wild fish populations (Kidd *et al.,* 2007; Overturf *et al.,* 2015).

ii. Target contaminants of emerging concern

For this study, 10 PPCPs comprising different classes as physicochemical properties were selected (Table 2.3): 17β-estradiol (E2), sulfamethoxazole (SMX), bisphenol A (BPA), ibuprofen (IBU), 17α-ethinylestradiol (EE2), oxybenzone (OXY), triclosan (TCS), diclofenac (DCF), caffeine (CAF) and carbamazepine (CBZ).

Table 2.3 Physicochemical properties of each contaminant of emergent concern used in this study. Source: https://pubchem.ncbi.nlm.nih.gov

Compound	Acronym	CAS	Chemical formula	Molecular weight (g/mol)	Solubility in water (mg/L)	K_H (atm-cum/mole)	pKa	Log Kow	Log Koc
Oxybenzone	OXY	131-57-7	$C_{14}H_{12}O_3$	228.25	69 [(a)]	$1.5x10^{-8}$	8.07	3.79	2.98
Diclofenac	DCF	15307-86-5	$C_{14}H_{11}Cl_2NO_2$	296.15	2,37 [(a)]	$6.1x10^{-8}$	4.15	4.51	2.39
Triclosan	TCS	3380-34-5	$C_{12}H_7Cl_3O_2$	289.54	10 [(b)]	$2.1x10^{-8}$	7.90	4.76	3.38 – 4.20
Caffeine	CAF	58-08-2	$C_8H_{10}N_4O_2$	194.19	$2.16x10^4$ [(a)]	$1.1x10^{-11}$	0.7/14.0	-0.07	2.87 – 3.89 [(e)]
Sulphamethoxazole	SMX	723-46-6	$C_{10}H_{11}N_3O_2S$	253.28	610 [(c)]	$6.4x10^{-13}$	1.60	0.89	1.85
Carbamazepine	CBZ	298-46-4	$C_{15}H_{12}N_2O$	236.27	17,7 [(c)]	$1.1x10^{-10}$	13.9	2.45	2.71
Bisphenol A	BPA	80-05-7	$C_{15}H_{16}O_2$	228.29	120 [(c)]	$4.0x10^{-11}$	9.60	3.32	2.06 – 3.59
17β-estradiol	E2	50-28-2	$C_{18}H_{24}O_2$	272.40	3,6 [(e)]	$3.6x10^{-11}$	10.3	4.01	4.48
17α-ethinylestradiol	EE2	57-63-6	$C_{20}H_{24}O_2$	296.41	11,3 [(e)]	$7.9x10^{-12}$	10.3	3.67	2.71
Ibuprofen	IBU	15687-27-1	$C_{13}H_{18}O_2$	206.29	21 [(a)]	$1.5x10^{-7}$	4.91	3.97	3.53

[(a)] at 25 ºC; [(b)] at 20 ºC; [(c)] at 37 ºC; [(d)] at 27 ªC; [(e)] silt - sandy loam soils

1. 17β-Estradiol (E2)

One of the main oestrogens released into the environment is the steroidal hormone estrone 17β-estradiol (E2) which is excreted by all humans and animals (Lucas *et al.,* 2006). It has been shown that exposure to oestrogen levels as low as 1 ng/L (<10 pM) is enough to cause severe impacts in some animal species, like the feminization of male trout (Hansen *et al*., 1998) and the development of intersex roach in rivers (Jobling *et al*., 1998). The removal of this substance from 18 Canadian municipal treatment systems were examined by Servos *et al.,* (2005), in general E2 was removed effectively, >75% and as high as 98%, in most of the conventional mechanical treatment systems with secondary treatment. In conventional activated sludge and lagoon treatment systems, the mean concentrations of E2 and estrone in influent were 15.6 ng/L (range 2.4–26 ng/L) and 49 ng/L (19–78 ng/L). In the final effluents, the mean concentrations of both E2 and estrone were reduced to 1.8 ng/L (0.2–14.7 ng/L) and 17 ng/L (1–96 ng/L), respectively.

A study performed by Lucas *et al*., (2006) determined that the rate of this hormone mineralization was strongly influenced by both soil type and the matrix in which the hormone was added to the soil. It is clear from this study that in comparison to many xenobiotics, the oestrogens, estrone and E2 are not particularly persistent in soil especially when present in a natural matrix lasting only a few days in most soils.

Another research by Mashtare (2013), concluded that, in general, E2 was found to exhibit moderate to moderately-high sorption, degrade rapidly in the aerobic environment, and persist longer under anaerobic conditions.

2. Sulfamethoxazole (SMX)

Sulfamethoxazole (SMX) is a worldwide used antibiotic, which is commonly prescribed in clinical treatment and veterinary medicine including farming and aquaculture. Due to the characteristics of SMX, approximately 15–25% is incompletely metabolized and excreted into the surrounding environment from the human or animal body after ingestion.

The removal efficiencies of SMX by conventional treatment plants vary significantly, depending on types of treatment processes and operation conditions. In 2008, Bhandari and co-workers, studied the concentration of SMX in WWTPs serving urban communities in the Midwestern United States. Concentrations averaged between 3.25-5.49 µg/L, which are similar to those obtained by other researchers, like Batt *et al.,* (2007) who reported concentration of 0.21–7.9 µg/L in treated effluents from China WWTPs.

Studies conducted in soils have confirmed that SMX is adsorbed by the organic matter and minerals of the clay fraction, but not by the sand fraction and gravels composing the soil, (Hou *et al.,* 2010; Barros *et al.,* 2018).

In 2010, a research conducted by Liu *et al*., in soil collected from an agricultural field in Conghua, southern China concluded that SMX dissipated more rapidly in non-sterile soil than in sterile soil, showing that biodegradation played a major role in the dissipation of SMX in the soil. The half-life of SMX was 4 days in non-sterile soil under aerobic conditions. Under anoxic conditions, its half-life in non-sterile soil was 11 days.

SMX contamination is of public concern because it can build up antibiotic resistance in bacteria and develop antibiotic resistant infections in humans by contacting resistant organisms or by having resistant microbes in the body (Yao, 2018).

3. Bisphenol A (BPA)

Bisphenol A (BPA) is manufactured in high quantities for its use in adhesives, powder paints, thermal paper and paper coatings. Implicated in endocrine disruption, BPA is also used in the primary production of polycarbonate plastics and epoxy resins which are used in the plastics industry (Mohapatra *et al.,* 2017; Pookpoosa *et al.,* 2014).

Regarding BPA concentrations in WWTP, several studies have been done in several countries. For example, in Quebec Mohapatra e*t al.,* (2017) showed that BPA was present in significant quantities (0.07 µg/L to 1.68 µg/L in wastewater and 0.104 µg/g to 0.312 µg/g in wastewater sludge) in the WWTP. The treatment plant is efficient (76%) in removal this pollutant from process stream. However, environmentally significant concentrations of 0.41 µg/L were still present in the treated effluent.

Another comprehensive study involving samples from twenty-five WWTPs, had median BPA concentrations in influent and final effluent of 400 ng/L and 150 ng/L, respectively. Median removal efficiencies ranged from 1 to 77%. Respective median BPA levels in primary sludge, secondary biological sludge, and biosolids were 230, 260, and 460 ng/g with digested biosolids having the highest concentrations. In the considered scenarios biological aerated filter and membrane bioreactor processes showed the best performance, while chemically-assisted primary treatment achieved the lowest removal (Guerra *et al.,* 2015). Similar results were obtained in five municipal WWTP in and around Bangkok (Thailand) the BPA levels in the effluents ranged between 57.5 ng/L and 257.0 ng/L (Pookpoosa *et al*., 2014).

In the environment, most of the BPA (78 to 99%) is fixed in the soil. Primary sources of BPA to terrestrial soils include the application of sewage sludge, irrigation with wastewater effluent, discharge of landfill leachate disposal and recycling of electronic waste (Huang *et al.,* 2014).

Once BPA has reached the soil, it is relatively immobile due to its high soil–water partitioning coefficient and can form non-extractable residues in 3 days. Sorption to soils and sediments is highly reliant on organic matter concentrations and particle grain size. Ionization of BPA could occur in extreme soils if pH values approach its pKa, which could result in enhanced leaching or percolation to groundwater (Corrales et al., 2017).

4. Ibuprofen (IBU)

Ibuprofen (IBU) is a non-steroidal anti-inflammatory drug used in the treatment of rheumatic disorders, pain, and fever. As one of the most widely consumed pharmaceutical drugs in the world it reaches the environment because it is excreted unchanged in urine (González-Naranjo *et al.,* 2013). The entry routes of this type of pharmaceutical compound into the environment are related with WWTPs, septic tanks, hospital effluents and animal excreta (Estevez et al., 2014).

Concerning its presence in WWTPs, previous research showed that IBU was not completely eliminated in WWTPs with removal efficiency ranging from 13 to 99%. It is detected at µg/L, and even effluents seem to contain it quite frequently at concentrations up to 100 µg/L (Picó *et al*., 2017; Hiller *et al*., 2017).

Studies conducted by Santos *et al.,* (2008) and Davarnejad *et al.,* (2018) identified IBU has existing in sewer wastewater in considerable amounts, up to 10 ng/L and 169 µg/L. In England and Wales, IBU even exists in surface and drinking water in the range of 0.025 to 0.475 mg/L. Another research with the aim to evaluate the occurrence of pharmaceutical compounds in wastewater, the influent and effluent, in fifteen WWTPs in five different regions of Portugal (North, Center, Lisbon and Vale do Tejo, Alentejo and Algarve), during the summer of 2013, found that IBU showed the higher concentration of about 0.995 mg/L in the effluent (Fortunato, 2014).

Smook *et al.,* in 2008, with the goal of discovering the best way to eliminate this type of compound in WWTPs, studied different types of removal in order to test its efficiency. More than 95% of ibuprofen was found to be removed in the aeration tank, with aerobic biodegradation being the dominant mechanism.

Application of wastewater in agriculture irrigation is one of the main entry ways of this compound in soil. The persistence of IBU in soils implies that it may behave conservatively once reaching subsurface soils where anaerobic conditions may be prevalent. Furthermore, although this pollutant can be subject to microbial degradation in surface soil under aerobic conditions both the poor adsorption and short residence times reported suggest that it may readily move downward (Lin and Gan 2011).

5. 17α-Ethinylestradiol (EE2)

As a synthetic oestrogen, 17α-Ethinylestradiol (EE2) is mainly used in birth control pills and other pharmaceuticals to treat the ailments of infertility, hormone imbalance, osteoporosis, among others. The generally low absorption efficiency of these pharmaceuticals, similar to what happens with other pharmaceuticals, will cause this chemical to be excreted and discharged into the environment, exhibiting potent biological effects at a very low concentration (ng/L) (Xu et al., 2015).

The treatment process in WWTPs is incomplete and insufficient to remove EE2 resulting in measured wastewater treatment plant (WWTP) effluent concentrations of up to 62 ng/L. Cargouët *et al.,* (2004) also reported that the highest level of free EE2 has been detected in domestic wastewater at 7 ng/L and in the effluent of wastewater treatment plants of up to 42 ng/L (Aris *et al.,* 2014). Although the concentrations observed in WWTPs studied by Lima *et al.,* (2012) were extremely low (ng/L) these compounds are extremely potent, particularly EE2 with an estrogenic potency about ten times that of natural hormones and has been detected in effluents of different sewage treatment plants (up to 94 ng/L).

Due to their hydrophobic properties, oestrogens are strongly sorbed into soils and can easily accumulate in soil and sediment (Yu *et al.,* 2018), potentially limiting the mobility and transport of oestrogens from soils to aquatic ecosystems where they seem to cause most damage (Hu *et al.,* 2018). Lima *et al.,* (2012) reported that Stumpe and Marschner (2007) considered that the electro-strong character of the benzene A-ring of the EE2 may be responsible for this strong sorption to organic matter.

Colluci *et al.,* (2001) reported somewhat longer dissipation rate constants for EE2 (0.1 to 0.37 d^{-1}) in moist agricultural soil microcosms. These rates correspond to half-lives between 0.5d and 7.7d. EE2 persisted in sterilized soil.

6. Oxybenzone (OXY)

Oxybenzone (OXY), also known as benzophenone-3, is an aromatic hydrocarbon that acts as an ultraviolet light filter, it is used in sunscreens and personal care products to help minimize the damaging effects of ultraviolet radiation (DiNardo & Downs, 2017). This type of chemical is also used in cosmetics, shampoos, fragrances, and flavours and as photo stabilizers in personal products and plastics (Schneider & Lim, 2019).

They are absorbed percutaneously and excreted in the urine, which enters the plumbing. However, approximately only 4% of the applied dose is absorbed through the skin, and excreted in the urine, leaving most of the filter on the skin to be washed off. This contaminant reaches the environment either indirectly via WWTPs or directly from swimming and bathing in lakes and rivers (Sánchez-Brunete *et al.,* 2011; Chen & Schröder, 2018; 2019; DiNardo & Downs, 2017). Due to their high lipophilicity water insolubility and relative stability against biotic degradation, they are expected to be found in wastewater and by using this water for irrigation in agriculture, they end up in soil (Sánchez-Brunete *et al.,* 2011).

Although OXY is removed with relative efficiency (68 to 96%) in conventional WWTPs (Balmer *et al.,* 2005), concentrations in WWTP influents and effluents ranged from 0.58 to 10.4 ppb with the highest concentrations reported in a wastewater influent at San Diego County in the USA (Kim & Choi, 2014). Similarly, six WWTPs in southeast Brazil evaluated levels of OXY and observed (0.18 to 1.15 ppb) in both raw treated and chlorinated water, indicating that the compound was not removed completely by the water treatment process (DiNardo & Downs, 2017). Chen & Schröder (2018) observed that the widespread use of OBZ has led to its release into the environment and today it is one of the most frequently detected UV filters in surface water and wastewater. Their review also registered highest concentrations up to 1.395 mg/L of CBZ detected along Trunk Bay in Virgin Islands.

Another interesting point, relevant to the spread of this contaminant is the fact that UV filters have been identified in the Arctic, suggesting that water currents are dispersing the filters beyond their initial deposition (Schneider & Lim, 2019), which gives even more importance to the choice of appropriate treatment in WWTPs.

7. Triclosan (TCS)

Triclosan (TCS) is used as an antiseptic, disinfectant, or preservative in PPCPs such as hand soaps and shampoos, mouthwash, toothpaste, and cosmetics, and in household items such as cutting boards (Goodman *et al.,* 2018).

The primary route for triclosan to enter the environment after its use is through discharge of effluent from wastewater treatment plants and disposal of sludge on land. Research shows that triclosan has been detected in sewage effluents and sludge (biosolids) due to their incomplete removal during wastewater treatment (Liu *et al.,* 2009; Coogan *et al.,* 2007). Regarding TCS

concentrations in WWTPs effluents, the overall mean concentration found in North Texas by researchers Waltman *et al.,* (2006) was 0.11 mg/L (range 0.03–0.25 mg/L), and the monthly means exhibited a seasonal pattern with summer and autumn concentrations reaching higher values than winter and spring. Concentrations of TCS from two activated sludge WWTPs in Columbus and Loveland, Ohio, USA, were 0.24 and 0.41 mg/L. This apparent seasonal pattern may be explained by the increase of usage of products with this compound in warmer months and also due to reduced infiltration during dry summer months.

Sorption data obtained through Karnjanapiboonwong *et al.,* (2010) indicate that oestrogens, TCS, and CAF had a moderate to strong tendency to partition from water to soil in the environment and TCS had a strong tendency to sorb to both sandy loam and silt loam soils. This tests also permitted to understand that TCS would not be mobile in the environment.

Similar conclusions were found by the study of Ying *et al.,* (2007), suggesting that TCS tend to sorb onto soil or sediment in the environment and biologically have a slow degradation rate. Also, TCS can be degraded by microbial processes in soil under aerobic conditions but is highly resistant to biodegradation in soil under anaerobic conditions. Research conducted by Ying *et al.,* (2007) recorded no changes in concentrations of this compound in sterile soil within 70 days. Which suggest that no degradation of TCS by chemical processes occurred in the sterile conditions. Therefore, biological processes were the main responsible for any loss in the nonsterile soil within 70 days.

8. Diclofenac (DCF)

The extensive use of diclofenac (DCF) with approximately 940 tons of the drug being consumed globally on an annual basis (Zhang *et al*., 2008) as a non-prescription, anti-inflammatory drug in human and animal health care, and consequently the constant release of this compound, by body excretions into the environment, has raised a concern about its potential effects on nature, animal and human health (Facey *et al.*, 2018).

Humans and animals excrete free and conjugated DCF and its metabolites are directed to municipal wastewater (Vieno & Sillanpää, 2014). This pharmaceutical has shown to be globally present in municipal WWTPs effluents as well as in the aquatic environment (Zhang *et al.,* 2008; Langenhoff *et al*., 2013; Buser *et al.*, 1998). In a review study led by Vieno & Sillanpää (2014), the measured maximum concentrations of DCF in municipal wastewaters vary between 0.44 and 7.1 µg/L and the mean concentrations between 0.11 and 2.3 µg/L. The paper analysed data from 18 different countries: Austria, Canada, China, Finland, France Germany, Greece, Pakistan, South Korea, Spain, Croatia, Sweden, Switzerland, Taiwan, UK and USA. In the previously cited article, by Fortuna & Costa (2014), regarding wastewater treatment efficacy in fifteen Portuguese WWTPs, DCF was the pharmaceutical compound that presented the lowest removal rate among the remaining drugs under study, with a mean percentage of 63% removal and a mean value of 0.0249 µg/L. Therefore, these effluents represent a significant source of DCF entering the

environment, especially into the soil (Margon *et al*., 2009). This compound has shown to cause harmful effects on organisms and to bioconcentrate in fish and mussel at environmentally relevant concentrations (Vieno & Sillanpää, 2014; Chen *et al.,* 2015).

The research of Al-Rajab *et al.,* (2010) concluded that DCF was rapidly mineralized without a detectable lag in three different textured agricultural soils: sandy loam, loam and clay loam, all incubated at 30°C, with this and other tests suggesting high biodegradation potential of DCF (Suarez *et al.,* 2010).

9. *Caffeine (CAF)*

Caffeine (CAF) is an alkaloid whose basic structure is purine and exists widely in the leaves, seeds and fruits of a large number of plants. Among them, cocoa beans, tea, coffee, cola nut and guarana are the best known. It is extensively used in non-alcoholic beverages and also in pharmaceuticals preparations as an adjuvant to drugs like paracetamol (Lakshmi & Nilanjana, 2013; Dash & Gummadi, 2006).

This alkaloid is frequently detected at significant concentrations in municipal sewage treatment plants, and in rivers receiving discharge, indicating that it is incompletely destroyed during the sewage treatment process (Hendel & Chapman, 2006). WWTPs are able to essentially completely remove CAF before discharging into the environment (Busse & Nagoda, 2005). The majority of the CAF (51-99%) is removed during secondary treatment, where biological processes are often stimulated with the presence of oxygen (Thomas and Foster, 2005; Sui *et al.,* 2010). CAF concentrations in influents and effluents of Swiss wastewater treatment plants (0.03−9.5 µg/L, respectively) coincide with such observations, showing an elimination of 81−99.9%. Despite the efficient removal in most WWTPs, caffeine was ubiquitously found in Swiss lakes and rivers (6−250 ng/L) suggesting that untreated sewage is overflowing into the water and that CAF is so abundant and chemically stable that it remains detectable (Buerge *et al.,* 2003).

Soil is a primary environmental compartment receiving CAF through wastewater irrigation and fertilization application. Williams & McLain (2012), observed that the average CAF concentration after 3 years in the 0- to 5-cm layer was 1.2 ng/g soil, and the average concentration decreased with increasing depth from the surface (1.1 ng/g soil for 10–15 cm; 0.6 ng/g soil for 25–50 cm). A study by Hendel *et al*., (2006) evidences that the mechanism of CAF dissipation in soil is microbial biodegradation. Their results showed that CAF was stable in autoclaved soil (sterile soil), the biphasic accelerating kinetics of CAF mineralization in the loam and sandy loam soils suggest that a competent population was improved after a brief lag and also the response of CAF mineralization in the loam soil to variation in temperature and moisture is consistent with the activity of a mesophilic aerobic biodegrading population.

10. Carbamazepine (CBZ)

Carbamazepine (CBZ) is used for the treatment of seizure disorders, for relief of neuralgia, and for a wide variety of mental disorders. Approximately 72% of orally administered CBZ is absorbed, while 28% is unchanged and subsequently discharged through the faeces (Zhang *et al.,* 2008).

The fate of CBZ and five of its metabolites were assessed from five WWTPs in Berlin, Germany. The parent compound CBZ was considered to be very persistent during wastewater treatment. In five out of the six samples, the concentration of CBZ even increased by an average of 14% during treatment (Bahlmann *et al.,* 2014). The same researchers, in Portugal, detected a median concentration of CBZ of 0.52 (0.03) mg/L from the two main WWTPs of Aveiro ("North" and "South") in 2010. Another study accomplished by Sajwan *et al.,* (2014) where water samples were collected from various water resources and five WWTPs from Georgia to study CBZ contamination levels. Measurable levels of CBZ were detected in all samples analysed and concentrations of CBZ ranged from 11.4 ng/L to 35.2 ng/L (WWTP effluent). The CBZ levels in effluent of WWTP were about two times higher than all other sites (range: 26.6-35.2 ng/L).

CBZ is a pharmaceutical with great environmental significance due to its limited removal efficiency during wastewater treatment processes and its high stability in the environment (Navon *et al.,* 2011). The removal efficiency of CBZ in WWTPs is mostly below 10% (Zhang *et al.,* 2008). CBZ has been reported to occur in surface waters, groundwater and treated wastewater effluents up to 6.3 µg/L and in biosolids up to 258 µg/kg (McClellan & Halden, 2001; Kahle *et al.,* 2009).

According to Shao *et al.,* (2018) only approximately 5.82–21.43% of CBZ degraded in all different studied soil settings, with changes in the presence of composted sewage sludge during an incubation period of 120 days.

2.3 Electrokinetic process

2.3.1. Principles overview

Electrokinetic (EK) remediation is a technology developed in the late 80s of the 20th century for the remediation of soils and other solid materials (Cameselle *et al*., 2013). This technique, also named electroreclamation, electrokinetic soil processing, and electrochemical decontamination, uses low level direct current (DC) on the order of mA/cm^2 across electrode pairs that are implanted in the ground generally on each side of the contaminated soil mass (Acar & Alshawabkeh, 1993; Shenbagavalli & Mahimairaja 2003).

This current simultaneously initiates many physical processes (heating, changes in viscosity, etc.), electrochemical processes (water oxidation and reduction, etc.), chemical processes (ion exchange, dissolution of precipitates, etc.) and electrokinetic processes (electro osmosis, electromigration, electrophoresis, etc.), which significantly change the soil (Rodrigo *et*

al., 2014). The main goal of this type of remediation is to affect the migration of subsurface contaminants in an imposed electric field via electroosmosis, electromigration and electrophoresis (Virkutytea *et al*., 2002).

Electroosmosis describes the movement of soil moisture or groundwater from the anode to the cathode of an electrolytic cell (Virkutytea *et al*., 2002). When DC is applied cations are driven towards the cathode and anions towards the anode. As both migrate, they carry water and they exert a viscous drag on the pore fluid round them (Mitchell, 1991; Ribeiro *et al*., 1999). This mechanism prevails in the removal of uncharged or weakly dissociated organic contaminants, like phenols. This component of transport is almost inexistent in coarse sands and high plasticity clays with lower water content (Ribeiro and Rodríguez-Maroto, 2006).

Electromigration refers to the movement of ions under the action of an electric field. It is the dominant transport mechanism in soils when dealing with charged soluble species (Ribeiro *et al.,* 1999). Positive ions are attracted and move toward the negatively charged cathode, and negative ions move to the positively charged anode (Virkutytea *et al*., 2002).

Electrophoresis refers to the movement of charged particles under an electric field (Acar & Alshawabkeh, 1999). The charged particles are attracted electrostatically to one of the electrodes and repelled from the other (negatively charged clay particles move in direction to the anode) (Ribeiro *et al*., 1999). In this manner contaminants bound to mobile particulate matter can be transported (Virkutytea *et al*., 2002). The described processes can be better comprehended through Figure 2.4.

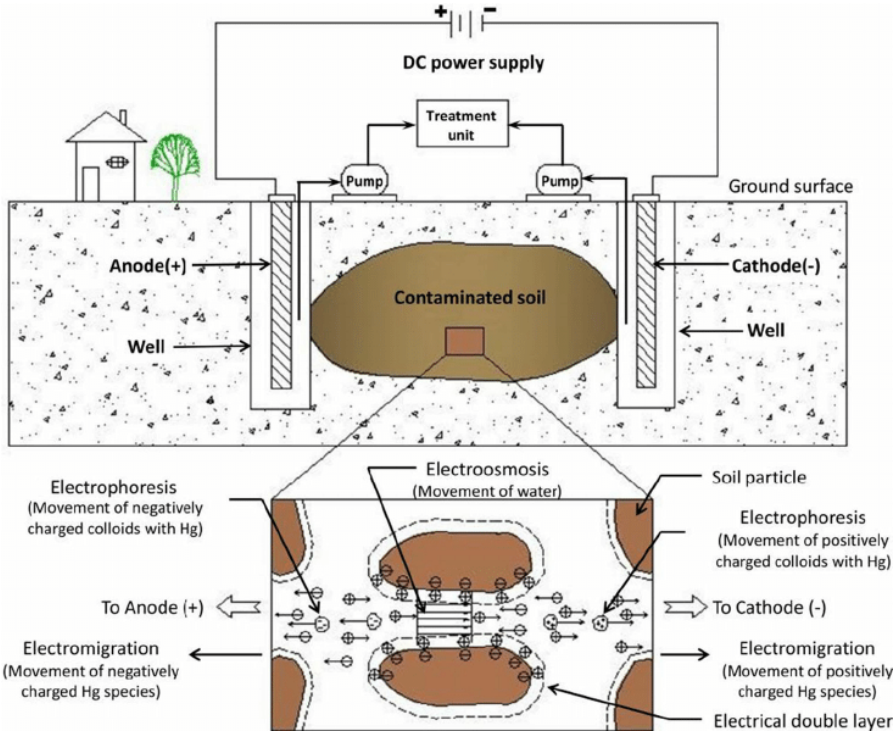


Figure 2.4 Scheme of EK remediation applied to a contaminated soil (Feng et al., 2015).

In recent years, the process has been enhanced having integrated chemical surfactants, chemical amendments and 101 chelating agents to enhance metals or organics desorption from soil to increase the contaminant removal rates at the electrodes or to degrade contaminants at their source (Lima *et al.,* 2017). The direction and quantity of contaminant movement is influenced by the contaminant concentration, soil type and structure, and the mobility of contaminant ions, as well as the interfacial chemistry and the conductivity of the soil pore water (Francisca *et al*., 2012). Because of that, mechanisms and their effects can be altered in order to enhance the removal efficiency or lock in non-critical contaminants in the soil by immobilization.

2.3.2. Periodic electric potential application

Applying periodic electric power, with time intervals where voltage is not applied, allows high electroosmotic flow and promotes enough time for mass transfer or the diffusion of the contaminant from the soil matrix to occur and thus its removal (Maturi et al 2009; Cameselle, & Reddy, 2013), with the additional benefit of saving in electric power consumption.

As stated by Cameselle & Reddy (2013), in the switch off intervals, the contaminant transfer from the solid (soil) to the liquid phase (interstitial fluid), as well as diffusion of the contaminant through the soil pores. This periodicity generates an electric current that follows an up-and-down pattern, and also when the voltage is switched off causes a higher current when the voltage is applied again (Ammami *et al.,* 2015).

An investigation with the goal to determine the contaminant mass removal using a periodic voltage application, accomplished by Reddy & Saichek (2004), concluded that considerable contaminant removal could be achieved by using a voltage gradient of 2.0 VDC/cm alongside a periodic electric potential application. The periodic voltage application consisted of a cycle of five days of continuous voltage application followed by two days where no voltage was applied. Results from these experiments showed that the test where a periodically voltage was applied, resulted in a much more sustained flow rate, that sustained for over 250 days with an average electroosmotic flow rate of over 7 mL/day, while another test in the same conditions but with continuously applied voltage, had diminished flow after less than 25 days, and although had high initial average flow rate of roughly 13 mL/day during the first 25 days, the average flow rate reduced to less than 1 mL/day.

This consistent flow largely contributed to the high amount of contaminant removal, and also due to the solubilization and kinetic desorption reactions and/or the pulsing mechanisms, caused by the use of the periodic mode of voltage application (Reddy & Saichek, 2004).

2.3.3. EK coupled with bioremediation

Numerous field studies have proven the commercial viability and technical effectiveness of EK remediation (Rodrigo *et al*., 2014; Guedes *et al.,* 2014; Cameselle & Gouveia, 2008; Kim *et al.,* 2001; Lopes, 2018; Lima *et al.,* 2017). However, the usual EK technique may not have an efficient removal or migration of contaminants. To overcome EK limitations and increase its efficiency, enhancement techniques or their combination with other technologies have been tested (Saichek & Reddy, 2005; Saeedi *et al*., 2009; Yuan & Weng, 2004).

These techniques may be applied to solubilize or mobilize the contaminants, control or adjust soil pH in the suitable range for EK and transform, breakdown, or destroy the contaminants (Yeung *et al.,* 2011). Some of the techniques that may be applied with EK include the use of surfactant, chelants, complexing agents, controlling soil pH, bioremediation, permeable reactive barriers, and ultrasonication. Selecting the suitable combination technique depends on the soil type and on the contaminant or contaminants necessary to remove (Jamshidi-Zanjani & Darban, 2017).

The use of EK in combination with bioremediation vastly improves the efficiency over that of EK remediation alone in removing pollutants from soil.

Bioremediation requires environmental conditions which are favourable for the biochemical process and interaction between microorganisms, contaminants, nutrients and electron acceptors/donors (Gill *et al.,* 2014). Consequently, this process may occur in the subsurface environment, but not at a rate to mitigate risks at a particular site. In most cases, the biodegradation rate is slow. EK enhances the degradation rate by using the transport mechanisms associated with electrokinetics to deliver nutrients and/or to introduce new bacteria if the indigenous microorganisms are not capable of degrading the contaminant (Hassan, 2016).

EK remediation can significantly enhance nutrients delivery to indigenous bacteria, thereby providing a tremendous potential for cleaning contaminated soils including fine-grained soils, which are usually difficult to clean-up using conventional methods (Yeung and Corapcioglu 1994; Alshawabkeh 2009; Reddy and Cameselle 2009). Many studies have investigated the use of EK to improve the outcome of bioremediation (Yeung *et al.,* 2011).

According to Hassan (2016) EK-bioremediation processes can be divided into two main aspects: microorganism related factors such as the existence of nutrients, and the microorganisms' capability of surviving, persisting, and degrading the contaminant; influence of EK processes, such us electrolysis reactions, electric current, change in temperature, power for electrokinetics, the availability of power lines near the contamination sites and the cost of electricity.

Results have shown that by coupling these two techniques it is possible to achieve better degradation rate values. A research conducted by Azhar (2016) verified that EK-bioremediation did facilitate the removal of heavy metals, using the EK technique 40% to 52% of mercury was removed from the soil, but when this process was combined with *Lysinibacillus fusiformis*, it could

remove up to 78% of mercury. Another study performed by Guo *et al.,* (2014) showed how the degradation rate of petroleum in the BIO–EK test was significantly higher than that of EK process alone, indicating that the biological degradation was stimulated by electric field, and signifying a synergistic effect between biological degradation and electrochemical stimulation.

2.4. Analytical techniques

2.4.1. QuEChERS method

A more recent and now widely used sample preparation method named QuEChERS was introduced in Rome in 2002 by Anastassiades, Lehotay, Stajnbaher, and Schenck. The abbreviation QuEChERS stands for quick, easy, cheap, effective, rugged and safe, describing the advantages this original analytical methodology combining the extraction/isolation of pesticides from food matrices and extract clean-up over the traditional liquid–liquid extraction (Zhang *et al.,* 2012; Wilkowska & Biziuk, 2017).

Originally, the QuEChERS was introduced for pesticides residues analysis in high moisture fruits and vegetables, but more recently it is gaining significant popularity in the analysis of broad spectrum of analytes in huge variety of samples (Rejczak &Tuzimski, 2015).

According to Anastassiades *et al.,* (2003), some of the most important characteristics of this method are the ones described below:

- Rapid (8 samples in less than 30 min)
- Simple (no laborious steps, minimal sources of errors)
- Cheap (ca. 1 € per sample for the sample preparation)
- Low Solvent Consumption (10 mL acetonitrile)
- Practically No Glassware Needed
- Wide Pesticide Range (polar compounds, pH-dependent compounds)
- Extract in Acetonitrile (GC- and LC- amenable)

The original procedure consists in the extraction a homogenized sample by hand-shaking or vortex mixing with the same quantity of acetonitrile (ACN) to result in a final extract sufficiently concentrated to remove the need for solvent evaporation.

Small quantities of salts (4 g anhydrous magnesium sulphate, $MgSO_4$) are then added to the sample, with mixing, to initiate partitioning of the analytes between the aqueous residue and the solvent. After centrifugation or simple vortex mixing, which leads to seamless physical separation of the phases, clean-up and removal of residual water is performed at the same time by use of a rapid procedure, dispersive solid-phase extraction (*d*SPE), in which a primary–secondary amine adsorbent and more anhydrous $MgSO_4$ are mixed with the sample extract (Lambropoulou & Albanis, 2007).

The right selection of the extraction solvent is critical in QuEChERS, as it directly determines its efficiency. Although ACN is miscible with water, it can easily be separated from water by the salting-out effect and centrifugation. ACN is also responsible for higher recoveries and less interference than other solvents such as acetone and methanol while offering slightly better limit of detection and relative standard deviations (RSD) than acetone (Zhang *et al.,* 2012)

QuEChERS method has important advantages over most traditional extraction methods, since it enables high recovery rates for wide range of analytes and produces very accurate results thanks to the use of an internal standard for elimination of problematic commodity differences. Another important advantage of the QuEChERS is the fact that this technique has rapid character and high sample throughput, meaning through this method, a big batch of samples could be extracted in relatively short time interval, less than 60 min, by a single analyst. Due to low solvent consumption and absence of chlorinated solvents and a possible small waste generation, this method is also in accordance with so-called green chemistry (Rejczak & Tuzimski, 2015). Similarly, the need of using only basic laboratory devices make this sample preparation technique relatively inexpensive in comparison to most traditional extraction methods.

Since QuEChERS requires fewer steps this is very significant, as every additional analytical step complicates the procedure and is also a potential source of systematic and random errors (Wilkowska & Biziuk, 2017). The scheme of the Figure 2.5 synthetizes the steps needed to accomplish this technique.

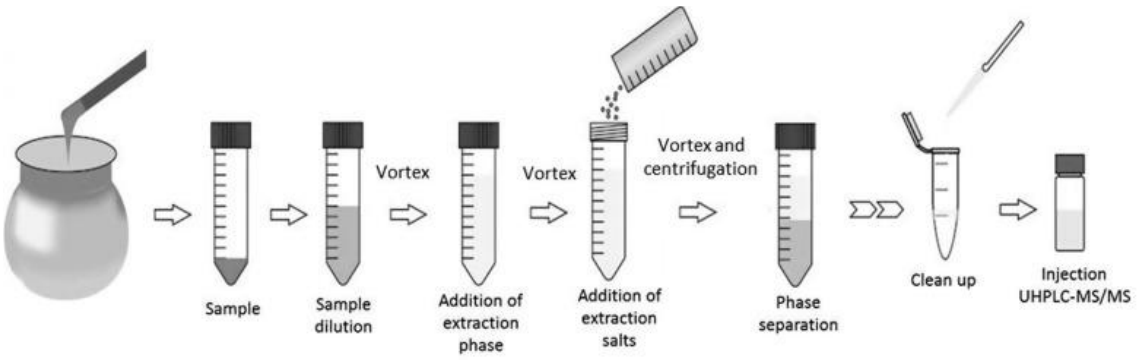


Figure 2.5 QuEChERS method adapted for the determination of pesticides (Source: Tette et al., 2016).

2.4.2. High Performance Liquid chromatography

Chromatography is a technique to separate mixtures of substances into their components based on their molecular structure and molecular composition. It involves a stationary phase (a solid, or a liquid supported on a solid) and a mobile phase (a liquid or a gas), the latter, flows through the stationary phase and carries the components of the mixture with it. Components with weaker interactions will move faster through the column than those that display stronger interactions with the stationary phase. This difference in rates cause the separation of different components (Giri, 2019).

Chromatographic separations can be carried out using a variety of stationary phases, including immobilized silica on glass plates (thin-layer chromatography), volatile gases (gas chromatography), paper (paper chromatography) and liquids (liquid chromatography) (Giri, 2019).

High performance liquid chromatography (HPLC) is an application of liquid chromatography. HPLC guarantees a high sensitivity, and, simultaneously, this technique has its gas analogue. The principle of HPLC is the same as that of liquid chromatography, liquid–solid chromatography and liquid–liquid chromatography. The stationary phase may be a solid or liquid on a solid support. The mechanisms responsible for distribution between phases include surface absorption, ion exchange, relative solubilities and steric affects (Aniszewski, 2007).

There are many different stationary and mobile phases that can be used in HPLC, and for this reason there is a great variety of separation mechanisms. Studies on HPLC use different criteria in their attempt to classify the modes of this technique: type of stationary phase, predominant separation mechanism, type of groups of compounds it is aimed at. These criteria sometimes overlap; which means that is possible to work in several different chromatographic methods with the same stationary phase, and the same group of compounds can be separated using several different stationary phases, and so it is challenging to classify HPLC techniques into specific groups (Moreno-Arribas & Polo, 2003).

A pump working with high-pressure leads the mobile phase from a reservoir through an injector. The mixture under analyses is injected into the column, passed through the particle bed, for component separation and after, the separated mixture moves into the detectors, as a mobile phase, where the absorbance is monitored by one or multiple detectors (e.g. diode array detector (DAD) and fluorescence detectors (FLD)) (Lopes, 2018).

A single solvent is often used to carry out the separation (isocratic elution) but differing proportions of various solvents are also often used (gradient elution). Today most chromatographs are controlled by a computer, that is also used for data collection, making possible to achieve greater quality of quantitative data and enable automation of the system. (Moreno-Arribas, M. V., & Polo, 2003).

HPLC systems provide key benefits such as the possibility to control and automate chromatography instrumentation, provide data management, security features, and reporting and instrument validation, they are powerful and adaptable, increase productivity by managing all the areas of analysis - from sample to instrument, and from separation to reporting results and more affordable (Thomas, 2013).

3. Materials and methods

3.1. Soil sample

The soil was collected in an organic tomato plantation, located in São Nicolau, Santarém, Portugal (39°12'42.6"N 8°42'41.5"W) managed by Marco Nunes productions, in October 2017, the location is depicted in Figure 3.1.

The soil was collected between 0-15 cm depth, using a mattock and a stainless-steel shovel (coarse elements, roots, and tomato remains from previous crops were manually removed prior to sampling). Prior to use, the soil was sieved (No. 10 IS Sieve, 2.0 mm) to remove the coarse fractions, and its physiochemical characterization was undertaken at ex. *Estação Agronómica Nacional*, using standard methods (Lopes, 2018).

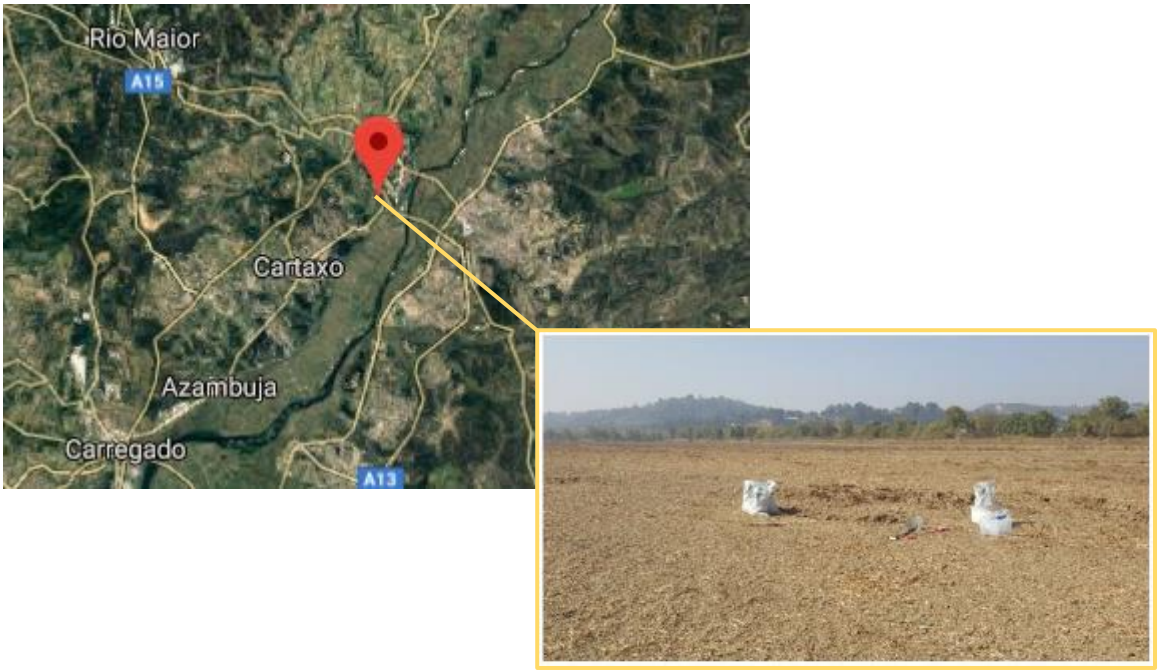


Figure 3.1 Location of the soil sampling and photograph of the field; São Nicolau, Santarém, Portugal (39°12'42.6"N 8°42'41.5"W: Google Maps, 2018; Lopes, 2018.

The experiments were conducted with non-sterile and sterile soil. The soil sterilizations were performed in a flow chamber under direct UV light for 2h. After, the soil was subjected to 6 times 1h cycles at 121ºC in an autoclave. To confirm a complete sterilization, the soil was extracted in 1% peptone water (1:100) under 3 cycles of 2 min of vacuum extraction followed by rest of 30 min at 30ºC. The extract (100 µL) was then used to inoculate petri dishes with malt extract agar and potato dextrose agar mediums. The inoculated petri dishes were placed in an

incubator at 30ºC and 60% humidity for 7 days and daily monitored; no microbiological community development was observed in the sterile soil.

3.2. Chemicals and solvents

The organic compounds (analytical standard) SMX, IBU, CAF, BPA, CBZ, DCF, EE2, were acquired from Sigma-Aldrich (Steinheim, Germany), TCS (≥ 97%) from Labesfal Pharma, and E2 and OXY from Alfa Aesar (Massachusetts, EUA). All used solvents (gradient HPLC grade) and reagents were acquired from Sigma–Aldrich (Steinheim, Germany), Panreac (Barcelona, Spain), Merck (Darmstadt, Germany), Carlo Erba (USA), J.T.Baker (Germany) or Fluka (U.S.A). The deionized water used was purified with a Milli-Q plus system from Millipore (Bedford, MA, USA). The safety data sheets of all standards and reagents used are presented in the annex 1. All produced residues (liquids and solids) were discarded to appropriate containers and treated according the FCT NOVA Internal Waste Disposal specifications and procedures.

3.3. Electrokinetic experiments

3.3.1. Electrokinetic set-up

All experiments were performed in a microcosm established in a rectangular acrylic container (100x50x70 mm) with two metal mixed oxide meshes (MMO) as electrodes (IrO_2/RuO_2 coated titanium, 20x970x1 mm; from Force) portrayed in Figure 3.2 (a). The MMO electrodes (inserted on each microcosm side at 5 mm from the edge) were connected to a power supply for direct current generation (Hewlett Packard E3612A, Palo Alto, USA).

In the abiotic conditions, all material used (e.g. soil, the acrylic cells, electrodes, Mili-Q water, glass material, micropipette tips, etc.) was previously sterilized at ITQB NOVA facilities. Before measuring the temperature and watering the cell, the tools and gloves of the user were sprayed with ethanol (70%) to ensure a sterile environment (Figure 3.2 (b)).

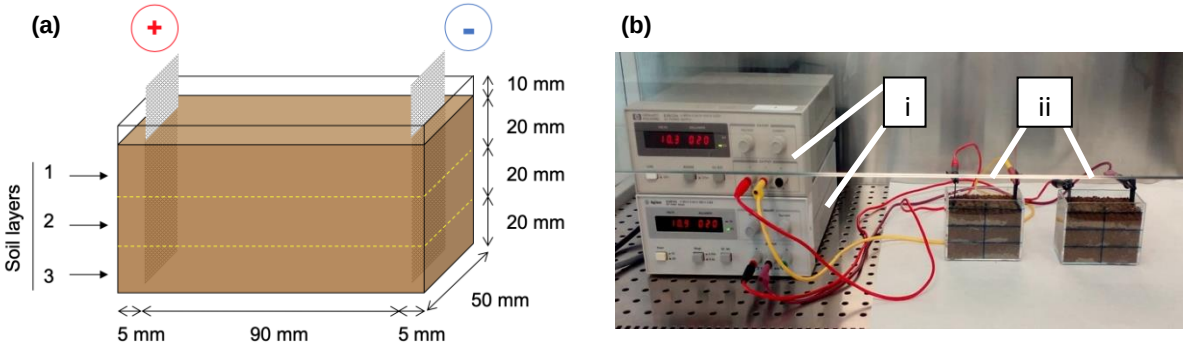


Figure 3.2 Experimental (a) microcosm scheme and (b) experimental setup used with power supplies (i) and electrodes and wires (ii).

3.3.2 Experimental design

To assemble the microcosms, the soil (total of 345 g dry weight) was poured in 3 consecutive layers in which the two bottom layers were watered with 25 mL of deionized water (layer 2 and 3 in Figure 3.2 (a)) and the top layer (layer 1 in Figure 3.2 (a)) was spiked with 25 mL of a solution containing the organic contaminants (each at 12 ppm), hereafter defined as the CECs mixture.

To remediate the soil containing the CECs mixture, each EK process (duplicate) was run for 4 days, in a flow chamber at 24ºC and protected from direct UV light. The EK was set to 20 mA with an ON/OFF switch of 12h, which consisted on the continuous application of identical periods of current and void. The experiments were performed with sterile (EK-Abiotic) and non-sterile soil (EK-Biotic) to assess the contribution of the current and biological community to the CECs remediation (Table 3.1). The effect of a lower temperature (room temperature at 18ºC) on the bioremediation alone and couple to EK was also assessed (Table 3.1). In parallel, the time zero control was also analysed in which the soil sections were processed immediately after spiking the soil with the CECs mixture, and three negative controls where no current was applied during four days in sterile and non-sterile soil at 24 and 18ºC (*i.e.* abiotic and biotic controls) (duplicates in all cases).

Each experiment was daily monitored for the current intensity, the voltage between the the working electrodes and the soil temperature. After four days, the 60 mm soil layer was carefully removed and segmented into 6 sections across distinct planes (Figure 3.3): transverse (layers 1, 2 and 3); tangential (anode section - A; cathode section - C); which were immediately processed and analysed for water content, pH, electric conductivity (EC) and organics contaminants concentration (see section 3.4).

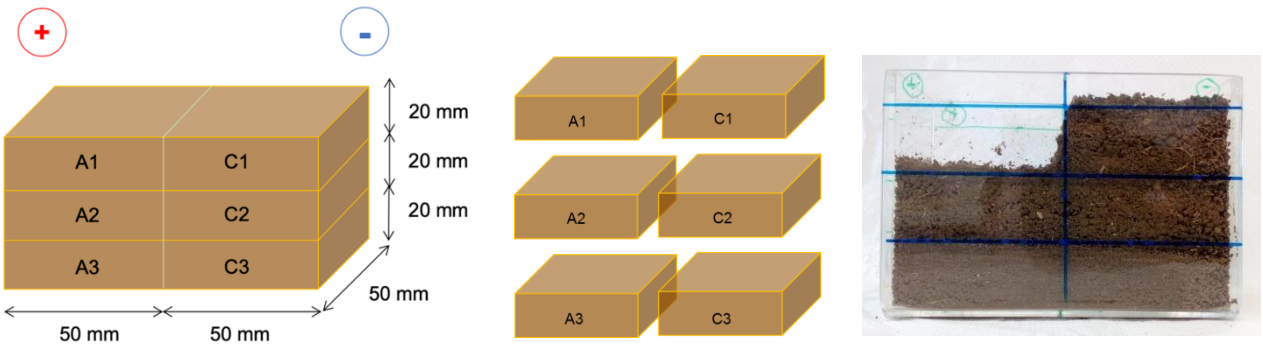


Figure 3.3 Soil division at the end of the experiments.

Table 3.1 Experimental parameters applied to each assay.

Experiment	Soil	Temperature (ºC)	Current (mA)	Variable tested
Abiotic-24	Sterile soil	24	-	Microbiota input to degradation
EK-Abiotic-24			20	EK input to degradation in a scenario without organisms
EK-Abiotic-24-W				Irrigation contribution to degradation process
Biotic-24	Non-Sterile	24	-	Microbiota input to degradation at a warmer temperature
EK-Biotic-24			20	EK input to degradation in a warmer set with living organisms
EK-Biotic-24- W				Irrigation contribution to degradation process
Biotic-18		18	-	Microbiota input to degradation
EK- Biotic-18			20	EK input to degradation in a scenario with living organisms

3.4. Analytical Methodologies

3.4.1. Physicochemical parameters

The soil temperature was measured with a digital thermometer that was cleaned with paper towel and methanol prior measurements.

The water content of the soil was measured as the weight loss after at 105ºC till constant weight and calculated according to equation 3.1:

$$\%\, Soil\ Water = \frac{weight\ of\ wet\ soil\ (g) - weight\ of\ dry\ soil\ (g)}{weight\ of\ dry\ soil\ (g)} x100 \qquad (3.1)$$

The pH and conductivity were measured using a soil deionised water ratio of 1:5 (w:v), stirred for 1h (approximately 100 bpm), and then left aside to settle for another hour prior measurement with a pH meter (Metrohm-Solitrode with Pt1000) and a conductivity meter (Horiba-LAQUAtwin).

3.4.2 Organic contaminants extraction

The concentration of the organic compounds was determined using an adapted QuEChERS method previously described in Pinto *et al.,* (2010). Briefly, the method is started by weighing and collecting 2.5 g of moist soil (collected right after the end of the experiment) into a 15 mL Falcon tube. Afterword 1.5 mL of deionized water is added to the soil and vortexed for a few seconds (approximately 30 seconds). Then 2.5 mL of acetonitrile is added, and the mixture is vortexed again for 1 minute. After mixing, 1 g of $MgSO_4$ is added and manually stirred vigorously for a few seconds followed by vortex for 30 sec. The samples are then centrifuged at 11000 rpm for 5 minutes (23°C) which results in a good separation between the solid matter and the liquid (Figure 3.4). The supernatant is then collected and filtered through polytetrafluoroethylene syringe filter 0.45 μm (previously passed through acetonitrile) and transferred to a vial. All soil sections were extracted and analysed in triplicate.

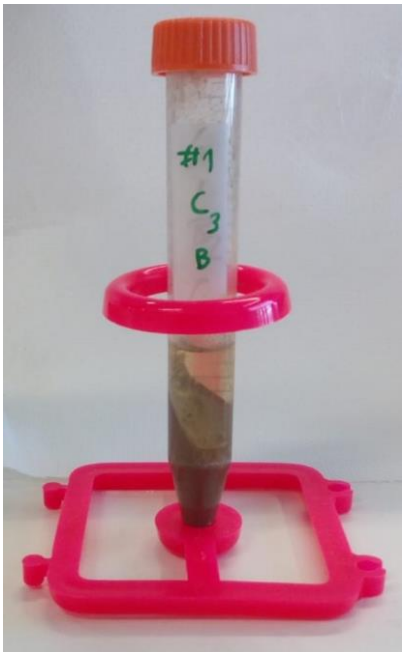


Figure 3.4 Sample in a falcon tube before collecting the supernatant.

3.4.2.1 HPLC analysis

To determine the concentration of the organic compounds in the soil samples, a high performance liquid chromatography (HPLC) with diode array detector (DAD) (G1315B) and fluorescence detector (FLD) (G1321A), both from Agilent 1 100 Series, was employed. The analysis was performed on the 1260 Infinity II LC Systems (Agilent Technologies, USA) equipped with a 1260 Infinity Quaternary Pump (G7111B) and an automatic sampler 1 260 (G7129A). Separation of the analytes was performed on a Poroshell 120 EC- C18 2.7 µm column with 4.6x100 mm from Agilent (California, USA), and an Onyx SecurityGuard C18 cartridges, with 5x4.6 mm, from Phenomenex (Torrance, USA).

The UV wavelength was set to full scan from 220 nm to 500 nm. Target compounds were measured at 282 nm for CAF, SMX, CBZ, DCF, OXY and TCS (channel DAD B), and 220-290 nm of excitation-emission for BPA, E2, EE2 and IBU (channel FLD A). All operations and data analysis were processed by the LC OpenLab software.

All HPLC runs were performed at a constant flow rate of 1.5 mL/min, in gradient mode. The eluents used were acetonitrile/Mili-Q water solutions (solution A: 35/65; solution B: 90/10), adjusted to pH = 2.8 using a formic acid solution (50% in water).

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 (Table 3.2). Post run equilibrium was carried for 2 min and the oven temperature maintained at 36 °C. Preceding analysis, 200 µL of sample extracts were mixed with 100 µL of eluent A (2:1) in a vial with insert and analyzed.

Table 3.2 HPLC gradient flow rate of 1.5 mL/min, in gradient mode. The eluents used were ACN/MiliQ water solutions (solution A: 35/65; solution B: 90/10).

Time (min)	A (%)	B (%)	Flow (mL/min)
3.0	95.0	5.0	1.5
20.0	5.0	95.0	
22.0	3.0	97.0	
25.0	3.0	97.0	
27.0	95.0	5.0	

3.4.3 Method Validation

3.4.3.1. HPLC calibration curve and limits

For calibration purposes, individual stock solutions of the organic compounds were prepared in acetone:methanol (1:3, v:v) and kept at 4°C. Working solutions were prepared by the adequate mixture and dilution of the stock solutions into methanol:eluent A (2:1,v: v).

Calibration curve was carried out using 7 stock solutions with different CECs concentrations (0.5; 1.5; 2.0; 2.5; 5.0; 7.5; 10 ppm) to determine the adjust between compound concentration and the corresponding compound area of the peak using linear regression.

For the HPLC method limit of detection (LD) was calculated through the residual standard deviation (Sx) multiplied by 3, for each compound. The limit of quantification (LQ) is the value of the LD multiplied by 3 (Guedes, 2015).

3.4.3.2. Methods recoveries and limits

Recovery assays were performed to validate the extraction method. The exact method of calculation is determined by the following equation 3.2:

$$Recovery\ (\%) = \frac{\frac{obtained\ value - real\ value}{real\ value}}{} \times 100 \qquad (3.2)$$

Where *real value* represents known concentration added in the present sample and the *obtained value* is the concentration that is obtained through HPLC analysis.

3.4.4. Statistical analysis

The statistical analysis was carried out using GraphPad Prism 7 software through an ANOVA test and a Tukey test. A significance level of 5% was considered (95% confidence interval, $p<0.05$) to apply the Tukey test. In order to validate the results obtained, all the experimental values of pH, EC, moisture content, organic contaminants degradation percentages and distribution within the microcosm were comparatively examined.

For the pH, EC, and moisture content the comparisons were carried out following three variables: (i) comparing to soil initial values; same experiment, different soil sections; (iii) different experiments, same soil section.

Compounds degradation percentages were analysed in two variables: (i) same compound, different experiments; (ii) different compound, same experiment. Compounds spatial distribution was analysed in three variables: (i) same compound, same experiment, different soil sections; (ii) same compound, different experiments, same soil section; (iii) different compounds, same experiment, same soil section.

For statistical purposes, samples which values were below MLD or MLQ (not detected, n.d.) were considered as zero. Cases that did not have SD associated (e.g. lost samples), were not considered while evaluating statistical differences.

4. Results and Discussion

4.1. HPLC method validation

The equation used is defined by a linear regression formula $y=mx+b$, y being the peak area, m the slope, x the concentration of the compound in ppm and b the y-intersect. The calibration curves that resulted from the regression are presented in the Table 4.1, as well as the retention times, determination coefficients (r^2), detection (LD) and quantification (LQ) limits of each compound. As shown in Table 4.1, most of the fits were excellent with correlation coefficients r^2 = 1.000, suggesting that the PPCPs calibration curve could be well described by a linear regression model.

Table 4.1 HPLC-DAD-FLD method validation parameters.

Compound	Detection challenge	Wave length (nm)	Retention time (min)	Calibration Curve	r^2	LD * (mg/L)	LQ ** (mg/L)
CAF	DAD-B	282	2.8	y = 20.4x - 0.47	1.000	0.13	0.39
SMX			4.5	y = 26.4x + 0.87	1.000	0.15	0.45
CBZ			5.7	y = 24.3x + 1.04	1.000	0.14	0.41
DCF			7.7	y = 17.1x + 0.80	1.000	0.18	0.55
OXY			8.2	y = 28.9x + 1.07	1.000	0.15	0.44
TCS			8.9	y = 8.31x + 14.8	1.000	0.16	0.48
BPA	FLD A	220-290[a]	6.3	y = 11.0x + 0.88	1.000	0.20	0.59
E2			6.5	y = 9.0x + 0.91	1.000	0.16	0.47
EE2			6.8	y = 8.6x + 0.86	1.000	0.19	0.58
IBU			7.9	y = 2.7x + 0.56	1.000	0.27	0.81

**LD=3Sx, where Sx is the residual standard deviation; **LQ=3LD;* [a] Emission-Excitation

4.2. Soil properties

The soil collected from an organic tomato plantation presented a clay texture class and the general properties can be found in Table 4.2. The soil, fraction <2.0mm, was extracted for PPCPs screening (n=6), through HPLC-DAD-FLD and GC-TOF-MS, and none of the organic compounds here under study were detected (<LD).

Clay soils generally have a higher cation exchange capacity than sandy or silty soil, as they are negatively charged and can attract, retain and exchange cations (Bohn, 1979). Because of this high cation exchange capacity (4-60 cmol/kg), very much dependent on the type of clay minerals presented, they are usually very fertile soils, and present a good nutrient bonding (Anthoni, 2000; Lopes, 2018). The clay particles have a large surface area *per* unit mass which allows the soil to hold a greater quantity of water and present low leaching level, particularly if the clay minerals are 2:1 type.

Table 4.2 Soil physical and chemical characteristics (soil fraction <2.0 mm).

Parameter	Value
Particle size distribution (%)	
Clay (ϕ < 0.002 mm)	61
Silt (0.002< ϕ < 0.02 mm)	29
Sand (0.002 <ϕ< 2mm)	10
Textural class: Clay	
Exchangeable cations (mg/kg)	
Ca	2245
Mg	402
Na	688
K	250
pH (H_2O)	8.06
Electrical conductivity (mS/cm)	0.71
Organic carbon (g/kg)	27
N organic (g/kg)	1.48

4.3. Remediation treatment effects on soil physicochemical parameters

EK phenomena and the success of EK-enhanced bioremediation treatment depends on environmental variables; therefore, adapting the treatment to the environment in which it is applied is important for managing electrode effects and predicting and sustaining EK phenomena (Gill *et al.,* 2014). Bioremediation requires environmental conditions which are favourable for the particular biochemical processes and interaction between microorganisms, contaminants, nutrients and electron acceptors/donors (Sturman *et al.,* 2004).

With that in mind, after the fourth and final day of each assay, the pH, conductivity and moisture levels were measured.

4.3.1. Temperature and moisture content

In order to study the effect of temperature in the remediation processes, two average temperatures were tested, 24°C and 18°C. The Table 4.3 displays the different set ups tested for each temperature and the mean temperature of each one.

No major temperature variations were observed in the soil, except in experiment EK-Abiotic-24 and EK-Abiotic-24-W, the data shows that soil temperature increased more than 3°C. This temperature increase may be related to the ohmic heating generated in the system by the electrical resistance of electrodes, the conductive elements, and of the soil itself (Ma *et al*., 2017). Electroheating may be used as an advantage since the increase of temperature and the final temperature in the soil can be easily controlled adjusting the intensity of the electric field. Furthermore, the heat is generated into the soil, in the whole volume at the same time, achieving a more uniform temperature in the area to be treated. The uniform temperature allows a uniform

removal of the contaminants and a more efficient use of the energy (Cameselle *et al*., 2013). Still, and although it is possible to observe slightly temperature fluctuations between experiments, no statistical differences were found (p>0.05).

Table 4.3 Average soil temperature of each experiment.

Room temperature (°C)	Experiment	Soil average temperature (°C)
24	Biotic-24	23.0 ± 0.1
	EK-Biotic-24	22.7 ± 0.1
	EK-Biotic-24-W	23.7 ± 0.1
	Abiotic-24	23.7 ± 0.1
	EK-Abiotic	24.5 ± 0.1
	EK-Abiotic-W	23.0 ± 0.1
18	Biotic-18	18.6 ± 0.1
	EK-Biotic-18	19.6 ± 0.1

The soil moisture may affect the electroremediation since it alters the electroosmotic flow rate and hence the decontamination of the soil by EK process (Ma *et al*., 2017), making it relevant to study the changes in soil water content. In general, the experiments present a trend of moisture increase from anode to the cathode section, being this a possible indication of electroosmotic flux (EOF) developing towards the cathode end (Lopes 2018; Hou *et al.,* 2018) and/or of a joule heating effect around the anode (Figure 4.1).

The lowest values of soil moisture were registered in the shallower surfaces of the microcosms, since they were the ones more exposed to evaporation and to the heat generated during the electrokinetic remediation, particularly in the anode compartment, reaching a low of 2.33% in section A1 of the EK-Abiotic-24 experiment. The highest value, 20.29%, was registered in section C2 in the EK-Abiotic-24-W assay, which was partially expected since this was an experiment with irrigation. This is corroborated by Tukey's multiple comparisons test (p<0.05), when comparing the same sections of the same experiment but with and without irrigation, the assay with no watering shows lower levels of moisture with a mean statistical difference of -15.14.

The Biotic scenario when subjected to the same conditions as the Abiotic, irrigation and DC, reveals much lower levels of water content, revealing a statistical difference of -11.46 between these experiments in section A1, -11.34 in section A2, -11.08 in section C1 and -11.15. in C2, Figure 4.1.

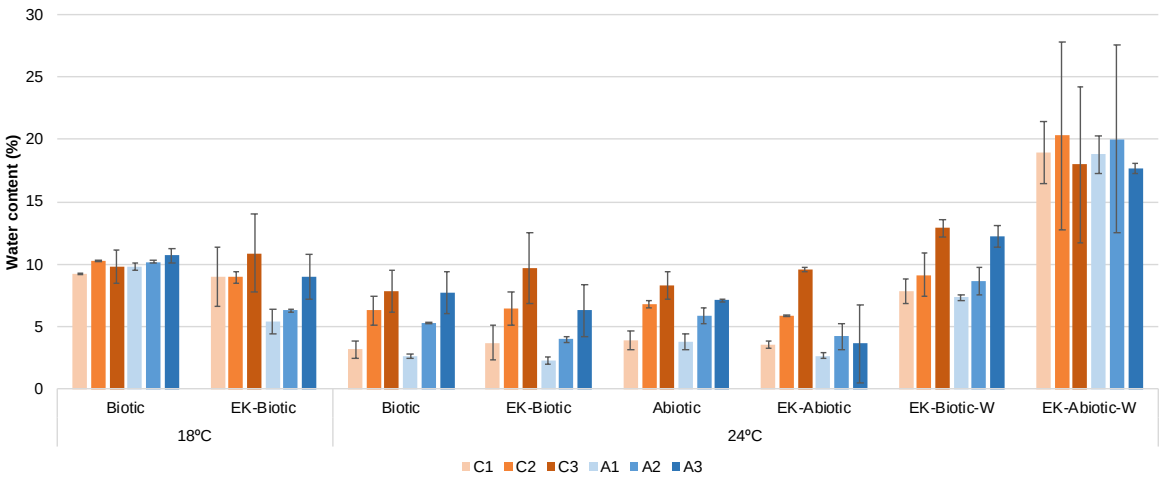


Figure 4.1 Water content (%) for the microcosms soil sections in all the experiments grouped by temperature, at the end of the 4 days.

4.3.2. pH and conductivity

Without applying DC, the pH values seem to be more constant showing a range of values from 7.84 to 8.10 in an abiotic setting, and from 7.89 to 8.27 when affected by living organisms.

When EK is applied, complex physicochemical processes generate non-uniform pH, voltage and moisture gradients that can affect bioremediation and, as proven in different studies, pH changes can alter the properties of some contaminants (McLean & Bledsoe, 1992). According to Daud *et al*., (2014), the pH of the soil system is a very important parameter, directly influencing sorption/desorption, precipitation/ dissolution, complex formation and oxidation/ reduction reactions.

Regarding pH, the lowest recorded level was 6.94 in EK-Abiotic-24, close to the natural pH value of the investigated soil (7.3±0.2), and the highest was 10.44 in EK-Biotic-24 experience (Figure 4.2). The cell sections that are situated in the cathode compartment are the ones which display higher levels of pH, particularly C2 and C3, whilst the lower levels of pH belong to the anode compartment and can be found in the A3 and A1 sections. The soil pH of EK-Abiotic-24 ranged from 6.94 near the anode to 10.44 near the cathode, making the pH fluctuate 3.5 units. In experiment EK-Abiotic-24-W, a difference of 2.61 between C2 and A2, and a difference of 2.4 between C3 and A3 in experiment EK-Abiotic-24-W was registered, displaying both, as held before, higher values in cathode. Hung *et al.,* (2018) reported analogous results, soil pH profiles along the EK cell illustrate that in a similar experience pH decreased to 3.4 near the anode side due to the H^+ produced from the electrolysis of water. Near the cathode side, the cell pH was increased to 10.5 because of the production of OH^- from water electrolysis.

Also, when analysing the pH variation spatially, there is an increase of these values with the depth, deeper sections have higher pH values for cathode, while in the anode the contrary occurs (Figure 4.2). In experiment EK-Abiotic-24 when compared section C1 to C3, there is a

difference of -1.6 in the mean, supporting the idea of increasing pH with depth in the cathode compartment. Regarding anode compartment the multiple comparison test (p<0.05) showed that deeper section of anode seems to have lower values of pH, which are also lower that the shallower sections of cathode. The analysis of EK-Abiotic-24 between section C1 and A1 and EK-Abiotic-24-W between section C2 and A2 demonstrate higher levels of pH for cathode compartment, registering a difference of 1.03 and 2.61, respectively. A bigger difference is attained when evaluating the section C3 and A3 in the same assay, EK-Abiotic-24-W, reaching a value of 2.4. Comparing to the respective controls (Biotic and Abiotic), a statistically significant difference of -0.92 in section C3, between experiment Biotic-24 and EK-Biotic-24-W, and of -1.7 in the same section, in similar experiments however without biota (Abiotic-24 C3 vs. EK-Abiotic-24-W C3) were observed. For easier understanding, the graphic 4.2 depicts the pH values for the different experiments.

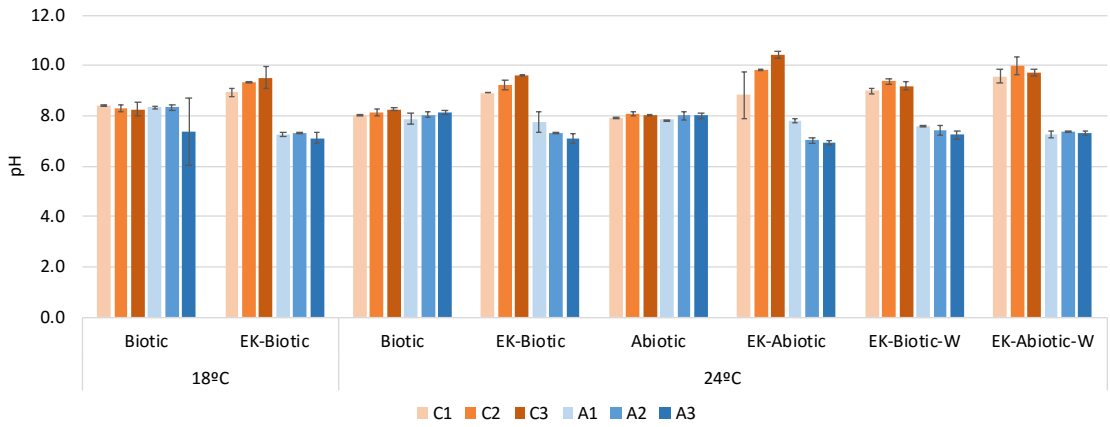


Figure 4.2 Soil pH for the different soil sections of the several performed experiments.

Soil electric conductivity (EC) as defined by Grisso *et al.*, (2009) is the ability of a material to transmit (conduct) an electrical current, affecting crop productivity, including soil texture, cation exchange capacity, drainage conditions, organic matter level, salinity, and subsoil characteristics. Since soil temperature influences EC readings, and temperature can fluctuate considerably in the upper 10cm of the soil during a day (Brevik and Fenton, 2003) but also because it has been shown that soil water and clay content can have the greatest influence on EC values, temperature and soil water content should also be considered when analysing EC.

The assays where no electric current was applied were the ones which displayed a more even range of conductivity, diverging from 0.60 mS/cm to 0.74 mS/cm in Biotic-18 and 0.54 mS/cm to 0.79 mS/cm in Biotic-24, revealing that temperature in these conditions does not severely affect the conductivity (Figure 4.3).

In general, in the assays with EK process, the highest values of conductivity were recorded in the sections C1 and A1, but mostly in the anode compartments, which show lower levels of pH. The statistical analysis verifies this too, having registered a statistically difference of -0.46 between sections C1 and A1 in the EK-Biotic-18 assay (p<0.05). The highest value of

conductivity registered was 1.05 mS/cm and it coincides with the lowest pH value obtained, both belonging to EK-Abiotic-24 experience. The lowest of 0.33 mS/cm was registered on the EK-Biotic-24-W near the cathode. According to Hou *et al.,* (2018) the oscillations of EC are related to the soil pH, and generally the EC of soil increases with the decrease of pH.

When evaluating the consequence of irrigation in the conductivity, comparing EK-Biotic-24-W to EK-Biotic-24, there is no noticeable change in the values, both displaying the same minimum of 0.33 mS/cm and a similar maximum of 0.79 mS/cm and 0.76 mS/cm, respectively. In the experiments with no biota present on the soil, EK-Abiotic-24-W and EK-Abiotic-24, the values showed a wider range but still similar, reaching 0.89 mS/cm and 1.05 mS/cm, being the lowest values 0.38 mS/cm and 0.33 mS/cm, respectively. Other studies, like the one performed by Costa *et al.,* (2014), show that higher EC values were obtained when the mean soil moisture content was higher and that there is a strong correlation between the two attributes.

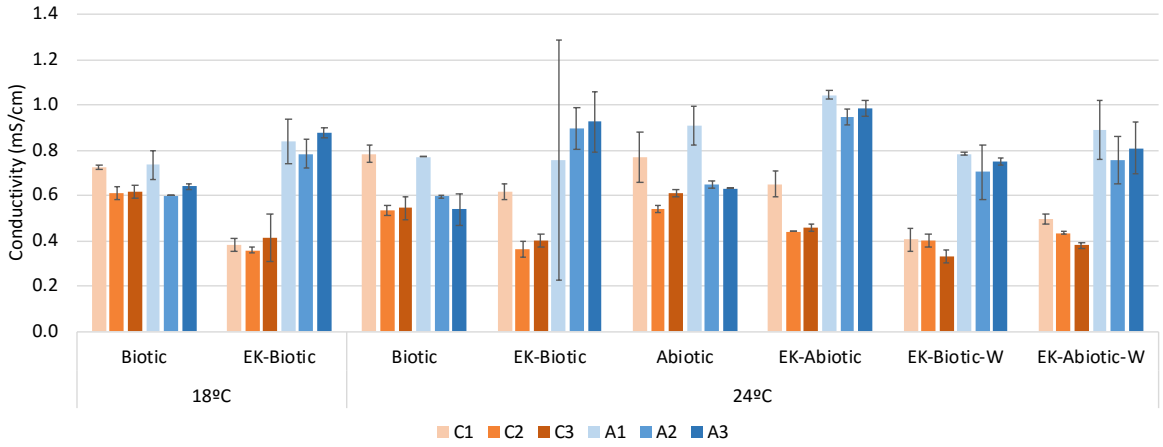


Figure 4.3 Soil Electric conductivity (mS/cm) for the microcosms soil sections in all the experiments grouped by temperature, at the end of the 4 days.

4.3.3. Voltage drop between the working electrodes

Assays without irrigation displayed a significance difference between their initial voltage values and the values achieved in the end. As shown in Table 4.4, EK-Biotic-24 was the experiment with the highest initial voltage with 17.35 V and oppositely, EK-Abiotic-24 had simultaneously the lowest initial value and the highest final value, 7.5 and 90.2 V respectively. In general, voltage was almost constant throughout the first three days increasing gradually.

At the fourth day, assay EK-Abiotic-24 increased by 145% and on the fifth day reached 90.2 V, which represented an augment in voltage of 82.7 V since day 1. EK-Biotic-24 and EK-Biotic-18, showed similar final voltage values 24.45 V and 21.25 V (approximately 10 V of increase), respectively, however, EK-Biotic-24 had an initial value 8.1 V superior to EK-Biotic-18 (9.25 V) as shown in Table 4.4.

Table 4.4 Voltage measured daily for the experiments with EK remediation without irrigation.

	Voltage (V)		
Days	EK-Biotic-18	EK-Biotic-24	EK-Abiotic-24
1	9.25 ± 0.01	17.35 ± 0.01	7.50 ± 0.01
2	10.05 ± 0.01	-	8.25 ± 0.01
3	12.45 ± 0.01	8.10 ± 0.01	11.50 ± 0.01
4	14.85 ± 0.01	15.50 ± 0.01	28.25 ± 0.01
5	21.25 ± 0.01	24.45 ± 0.01	90.20 ± 0.01

The voltage of the experiments in which the soil was watered every day were much more constant throughout the test than the previous three referred assays. The two tests show similar initial voltages, only differing by 0.25 V (Table 4.5). From the beginning of the experiments until the end, voltage increased slightly, and usually watering the soil resulted in a minor decrease, as displayed in Table 4.5. The augment of voltage from the first day until the last was analogous in two experiments, EK-Biotic-24-W had an increase of 3.3 V and EK-Abiotic-W-24 of 4.5V before irrigation and 1.95 after.

Table 4.5 Daily voltage before and after irrigation for the experiments EK-Biotic-24-W and EK-Abiotic-24-W.

	Voltage (V)			
Days	**EK-Biotic-24-W**		**EK-Abiotic-24-W**	
	before irrigation	**after irrigation**	**before irrigation**	**after irrigation**
1	7.8 ± 0.01	7.8 ± 0.01	7.55 ± 0.01	7.55 ± 0.01
2	9.7 ± 0.01	8.05 ± 0.01	10.3 ± 0.01	7.9 ± 0.01
3	11.3 ± 0.01	8.9 ± 0.01	10.95 ± 0.01	8.45 ± 0.01
4	12.2 ± 0.01	10.1 ± 0.01	12.05 ± 0.01	9.5 ± 0.01
5	11.1 ± 0.01	11.1 ± 0.01	12.03 ± 0.01	10.1 ± 0.01

4.4. Effect of remediation conditions on PPCPs removal from soil

Initially, all experiments were conducted inside the flow chamber at a room temperature of approx. 24 ºC. After 4 days, the total mass of contaminants (sum of all PPCPs mass under study) removed from the soil can be seen in Figure 4.4. In abiotic conditions (sterile soil) 6% of the total mass load of PPCPs was removed whereas the Biotic removals achieved 20%. Thus, we can infer that 14% of the achieved degradation is due to biodegradation processes (Biotic *vs* Abiotic).

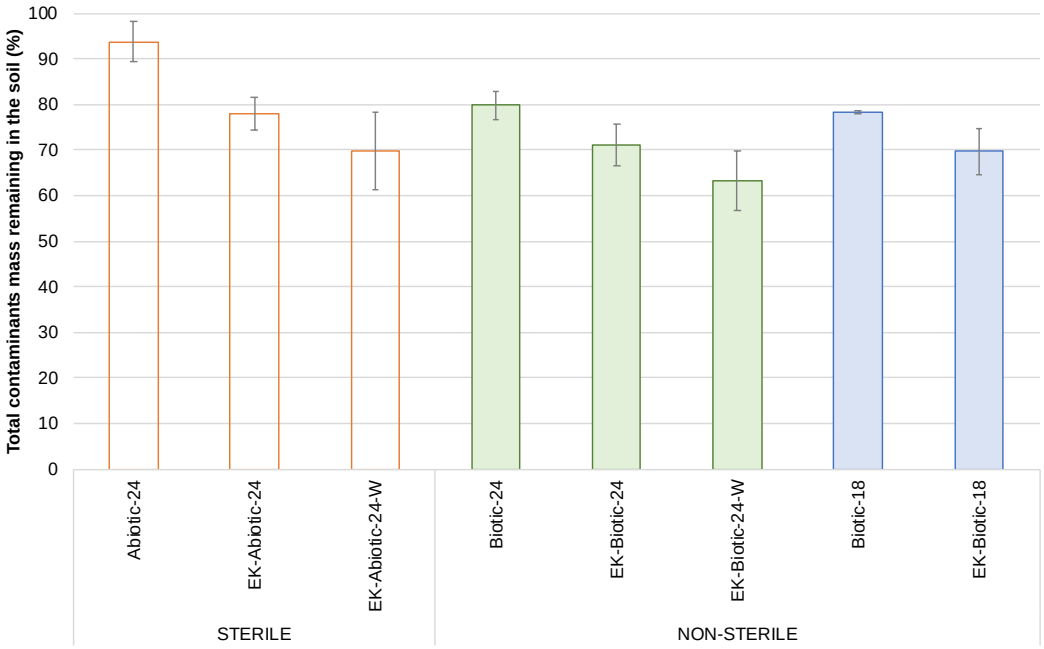


Figure 4.4 Contaminants mass (%) remaining in the soil after each assay grouped per *soil condition, sterile and non-sterile.*

Although the total mass removed from the soil was similar between the EK-Biotic and EK-Abiotic (Figure 4.4), the degradation *per* compound varied among experiments. For example, E2 degradation was always above 90% in the biotic conditions independently of the application of EK, but its degradation decreased to values below 33% in abiotic conditions (Table 4.6). Overall, the contaminant more susceptible to degradation was E2 (<LD) followed by BPA and SMX that reached 60% when the EK was applied in the Biotic conditions (Table 4.6). On the other hand, the compounds that are more recalcitrant to degradation in the conditions here studied were CBZ, OXY with an overall maximum degradation of 20%. Guedes *et al*., (2015) reported a similar result using sewage sludge, in which the compound that presented higher degradation was E2, followed by BPA, CAF and EE2, whereas IBU was not degraded. The results achieved for degradation were mainly attributed to bioremediation mechanisms as by the end of the experiment biological activity was observed in the collected samples (visual observation – a fungi growing in the soil was identified). Still, more microbiological analysis should be performed to corroborate these observations.

The application of an EK treatment allowed to decrease the total mass of contaminants in the soil to values below 70% when a daily irrigation was performed. A study conducted by Ferreira *et al.,* (2017) concluded that water content of the soil may play a significant influence on the desorption of the compounds and their consequently mobilization. This may be explained by the creation of larger interaction surfaces between soil and PPCPs that by increasing their dissolution help to release PPCPs that are bound in soil fraction.

The increase of degradation is slightly higher in the non-sterile setting, which may indicate that in this setting the coupling of electro-degradation with enhanced bioremediation was positive for decreasing pollutants loads.

Table 4.6 Percentage of compound degradation for each experiment.

RT (ºC)	Soil	Exp	Degradation (%)									
			E2	SMX	BPA	IBU	EE2	OXY	TCS	DCF	CAF	CBZ
24	Sterile	Abiotic-24	19 ± 3	14 ± 3	10 ± 4	5 ± 4	1 ± 3	1 ± 4	7 ± 3	5 ± 3	0 ± 2	0 ± 3
		EK-Abiotic-24	33 ± 1	43 ± 1	29 ± 6	19 ± 5	18 ± 1	9 ± 0	27 ± 4	14 ± 3	21 ± 6	6 ± 5
		EK-Abiotic-24-W	30 ± 7	81 ± 1	26 ± 5	37 ± 3	20 ± 6	18 ± 2	22 ± 8	28 ± 6	25 ± 4	14 ± 4
	Non-sterile	Biotic-24	90 ± 2	36 ± 7	29 ± 2	25 ± 3	13 ± 4	5 ± 4	0 ± 1	3 ± 7	1 ± 4	0 ± 4
		EK-Biotic-24	90 ± 0	51 ± 5	46 ± 2	19 ± 3	21 ± 7	9 ± 5	17 ± 4	16 ± 4	16 ± 3	4 ± 5
		EK-Biotic-24-W	100 ± 0	49 ± 6	67 ± 3	30 ± 5	41 ± 0	17 ± 6	0 ± 3	23 ± 5	27 ± 3	14 ± 2
18		Biotic-18	95 ± 1	45 ± 5	51 ± 5	16 ± 4	9 ± 1	0 ± 1	0 ± 7	0 ± 3	2 ± 4	0 ± 0
		Ek-Biotic-18	95 ± 0	65 ± 1	50 ± 4	12 ± 7	19 ± 4	15 ± 2	7 ± 4	10 ± 5	29 ± 8	2 ± 5

In the present study, the natural attenuation of each PPCPs, *i.e.* decay observed in the Biotic experiments, showed substantial differences as follows (Table 4.6): E2 (90%) >> SMX ≈ BPA ≥ IBU > EE2 > OXY ≈ DCF ≈ TCS ≈ CAF ≈ CBZ (0%). In the Abiotic conditions, the highest decay was 19% E2 followed by SMX ≥ BPA ≥ TCS > IBU ≈ DCF > EE2 ≈ OXY ≈ CAF ≈ CBZ.

Due to the complexity and variation in the process mechanisms and degradation of the compounds here under study, a deeper discussion will be performed as follows:

I. Overall assessment of the factors affecting PPCPs degradation
    a. Abiotic *vs* Biotic conditions
    b. Contribution of EK processes
    c. Influence of irrigation
    d. Influence of temperature

II. A deeper discussion *per* compound, starting on the more biodegradable ones followed by the more recalcitrant to degradation.

4.4.1. Factors affecting the PPCPs degradation

4.4.1.1. Contribution of abiotic and biotic processes

In general, the control conducted with sterilized soil (Abiotic-24) had lower levels of degradation compared to the non-sterilized soil (Biotic-24), which is shown in Table 4.7.

In sterile conditions at 24 ºC (Abiotic-24) the decay levels were very low, below 19% (Table 4.7). Previous studies have shown that the degradation of PPCPs in soil or sediment was influenced by microbial activities (Yu & Wu 2012; Ferreira *et al.,* 2017). Comparative experiments conducted by Yu *et al.,* (2013), in sterilized soils showed that the sterilization treatment resulted

in a decrease in the degradation rates of PPCPs. The average t1/2 of BPA increased from 13.0 to 17.2, indicating the contribution of microorganisms to BPA degradation. For CBZ and TCS, after incubating for 63 days, the average t1/2 in sterilized soils was 1.6 and 1.7 times longer, respectively. In the same study, it was settled that in sterilized soils, the concentration decreases, and the measured as decay was mainly attributed to aging and sequestration processes. Also, in 2014, Nam *et al.,* established that PPCPs behaviour vary from compound to compound and are difficult to predict, as their behaviour is often controlled by interactions with specific functional groups or complex pH-dependent speciation, which can help to explain the decay levels achieved.

Table 4.7 Degradation of PPCPs in the Abiotic and Biotic experiments conducted at 24 ºC (cells in light yellow reflect the highest value achieved for each contaminant, considering a minimum 5% difference).

Experiment	Degradation (%)									
	E2	**SMX**	**BPA**	**IBU**	**EE2**	**OXY**	**DCF**	**TCS**	**CAF**	**CBZ**
Biotic-24	90	36	29	25	13	5	3	0	1	0
Abiotic-24	19	14	10	5	1	1	5	7	0	0

PPCPs decay through volatilization from the moist soil surface is unlikely due to their low vapour pressures; the estimated Henry's Law constant for SMX and IBU are $6.4x10^{-13}$ and $1.5x10^{-7}$ atm-cu m/mole, respectively – lowest and highest values among the tested PPCPs (Table 2.3). In addition, in our setup their photodegradation should not be relevant, since the remediation experiments were conducted indoors and protected from direct UV radiation.

When a non-sterilized soil was used, E2, SMX, IBU and BPA compounds show higher degradation values compared to the abiotic conditions, more 71, 22, 20 and 19%, respectively (Figure 4.5). On the contrary, CAF, CBZ, DCF and OXY did not suffer biodegradation in the 4 days of this study (Table 4.7). Several studies have shown that biodegradation plays a major role in the removal of PPCPs from solid matrix (Karnjanapiboonwong et al., 2011; Xu et al., 2008; Rodarte-Morales et al., 2011). A study performed in 2013 by Yu *et al.,* showed that CBZ is one of the most persistent pharmaceuticals in the aquatic and terrestrial environment, with t1/2 ranging from 28.0 to 39.1 days, showing high persistence in soils due to its stable heterocyclic structure (Table 2.3). The t1/2 of BPA in soils in the same study were between 11.7 and 14.4 days, and more than 50% of BPA were degraded within 7 days. For CBZ and TCS, after an incubation of 63 days, the average t1/2 in sterilized soils was 1.6 and 1.7 times longer, respectively. A similar study conducted by Xu *et al.,* (2019), showed that after 45 days of incubation, soil concentrations of IBU and DCF were below their detection limits, attesting that some pharmaceuticals, can undertake fast degradation processes, showing low persistence in soils when exists biodegradation.

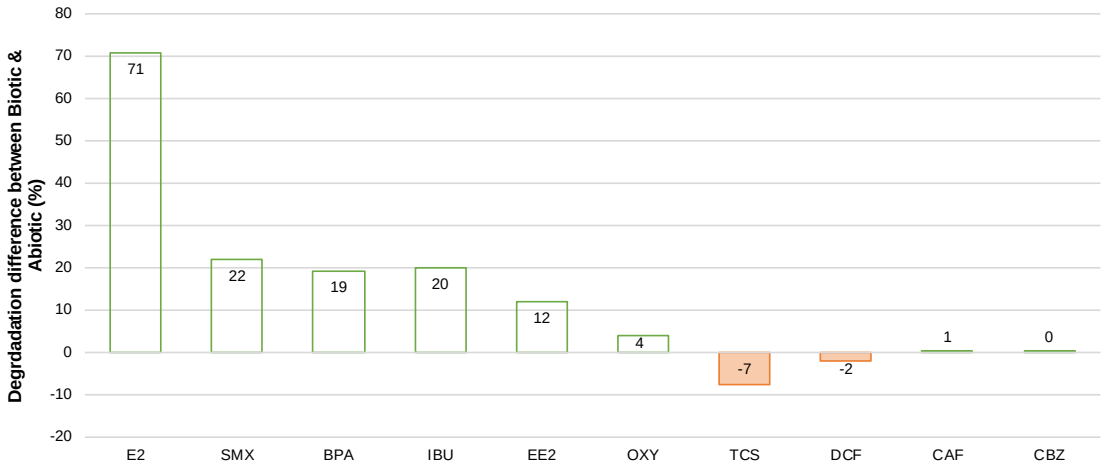


Figure 4.5 Biotic processes contribution to PPCPs degradation comparing to Abiotic processes (green bars show a positive impact of biodegradation comparing whereas the orange shows a negative impact).

4.4.1.2. Contribution of electrokinetic processes for PPCPs decay

When the EK process is applied to a contaminated soil it is expected that EK application increases the remediation by either:

(i) Enhancing bioremediation by making contaminants, nutrients, electron acceptors and electron donors more bioavailable to catabolically active microorganisms (Wick, 2009).

(ii) Or by promoting electro-degradation through mobilization by electroosmosis and stimulating a directed movement of pollutants in response to the presence of an electric current. The applied current produces hydrogen ions (H^+) at the anode and hydroxyl ions (OH^-) at the cathode, with a resulting pH gradient. Electromobile contaminants are therefore stimulated to migrate to positions where they are amenable to removal (Lear *et al.*, 2007).

As it can be observed in Table 4.8, higher degradation levels were achieved in the presence of soil indigenous microbiota, biotic conditions (non-sterilized soil) comparing to the abiotic conditions (sterilized soil).

Table 4.8 Degradation of PPCPs in the EK-Abiotic and EK-Biotic experiments conducted at 24 °C (cells in light yellow reflect the highest value achieved for each contaminant, considering a minimum 5% difference).

Experiment	Degradation (%)									
	E2	SMX	BPA	IBU	EE2	OXY	DCF	TCS	CAF	CBZ
EK-Abiotic-24	33	43	29	19	18	9	14	27	21	6
EK-Biotic-24	90	51	46	19	21	9	16	17	16	4

The biotic process contribution to this rate is considerable, as shown in Table 4.8. With presence of living organisms E2 and BPA show higher levels of remediation efficiency, more 57 and 17% comparing to EK-Abiotic (Figure 4.6). Maintaining the viability of an active degrader species (e.g. bacteria, archaea) is important for bioremediation in the natural environment, where microbial populations exist as diverse communities (Gill *et al.,* 2014).

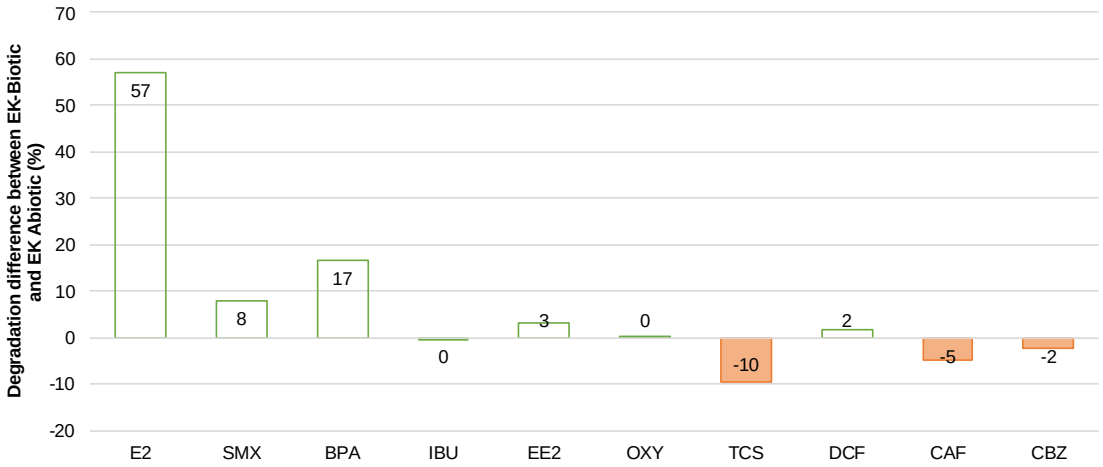


Figure 4.6 Influence of the biotic processes on EK applied to soil at 24 °C. The values represent the difference between the degradation values obtained for EK-Biotic-24 and EK-Abiotic-24 (green bars show a positive impact of biodegradation whereas the orange shows a negative impact).

The more recalcitrant contaminants such as CAF, CBZ, DCF, OXY and TCS show similar degradations in the EK treatments independently of the presence of soil microbiota. Thus, one can infer that these compounds degradation is mainly achieved through direct and indirect redox reactions promoted by the application of the low-level direct current (20 mA).

These compounds are environmentally recalcitrant and highly stable in soil, and during wastewater treatment. The biological degradation of CBZ and DCF was investigated under aerobic conditions by Thelusmond *et al*., (2018). While DCF exhibited rapid dissipation in all the aerobic soils, a limited decrease of CBZ was noted. Another study performed by Guedes *et al.,* (2015), concluded that independently of the cell design used, the application of a low-level direct current, improved the degradation of CAF between 20 and 47%, comparing against the control experiment (Biotic setting).

One possible explanation is that physicochemical properties of soils may also have contributed to the difference in PPCP degradation. Previous studies suggest that water content of the soil may play an important role on the desorption of the compounds and their consequently mobilization. This is attributed to the creation of larger interaction surfaces between soil and PPCPs that increase their dissolution helping to release PPCPs that are bound in soil fraction(s). In addition, the pH in the presence of soil is easily changed, which showed to be an important parameter in degradation/removal, as it increases the contaminant solubility (Ferreira *et al.,*

2017). In general, EK showed potential to enhance the degradation of the contaminants being the process mainly dependent on the intensity of the applied DC field and the cell design.

4.4.1.3. Influence of soil water content on removal efficiencies

As soil moisture content highly influences EK efficiency, a daily irrigation of the soil was carried out to see how it impacts the PPCPs removals. As described in Guedes *et al.,* (2014), in the specific case of organic contaminants, their removal by EK from the soil is mostly performed by electroosmosis.

In all experiments the same current was applied, 20 mA, however different levels of voltage were detected Table 4.4. In the EK-Biotic-18 assay, there was an increase in voltage from the initial mean value of 9.25V to 21.25 V in the fourth and final day, which might be explained by the water content on the soil that decreased (as referred in section 4.3.1; Figure 4.1). This is more obvious in the top layers that started to crack (Figure 4.7) as the soil dried due to the temperature (24 ºC) or due to water leaching to the bottom layers.

Taking this into account it felt pertinent to create a different set of tests which were watered every day with 20 mL of deionised water (type II) to evaluate the influence of moisture content in EK removal by increasing soil moisture and decreasing system resistance. The two experiments performed, coded EK-Biotic-24-W and EK-Abiotic-24-W, revealed that in general the contaminants reached higher degradation levels when the cell was watered and had the presence of microbiota (Table 4.6).

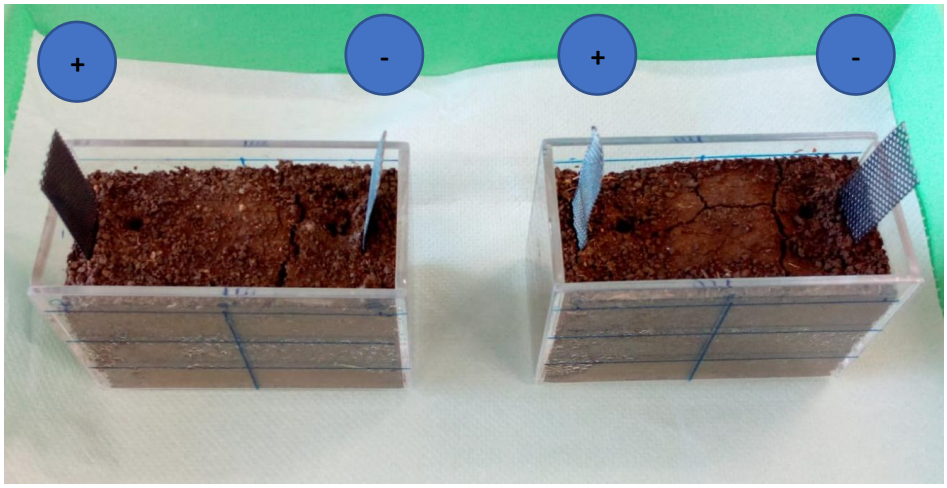


Figure 4.7 Photo of the microcosm after 4 days of EK treatment showing that the soil is dried and cracked.

Through the analysis of the Table 4.6 it is possible to conclude that not all contaminants display the same behaviour when under the same conditions. Some of the compounds, such as TCS, are more easily degraded in the presence of lower water content in the soil when in Biotic

conditions. On the other hand, E2, BPA EE2 are more easily degraded in the presence microorganisms in an irrigated system (EK-Biotic-W) but other pollutants such as CAF, CBZ, TCS seem to more easily degraded in an abiotic environment.

Table 4.9 Degradation of PPCPs in the EK-Abiotic and EK-Biotic experiments conducted with and without daily irrigation, at 24 °C (cells in light yellow reflect the highest value achieved for each contaminant, considering a minimum 5% difference).

Experiment	Degradation (%)									
	E2	SMX	BPA	IBU	EE2	OXY	DCF	TCS	CAF	CBZ
EK-Biotic-24	90	51	46	19	21	9	16	17	16	4
EK-Biotic-24-W	100	49	67	30	41	17	23	0	27	14
EK Abiotic-24	33	43	29	19	18	9	14	27	21	6
EK- Abiotic-24-W	30	81	26	37	20	18	28	22	25	14

4.4.1.4. Influence of temperature on electrokinetically enhanced bioremediation

As described by Killham (1994), increasing soil temperature to 30ºC is beneficial for microbial growth. For EK-Bio treatments it is an additional mechanism to enhance bioremediation, however, for EK applications close to the surface, ohmic heating may increase evaporation leading to reduced moisture content and increased electrical resistance (Virkutytea *et al*., 2002). To reach a better understanding of the influence of soil moisture and temperature in the contaminant's removal, a set of experiments were conducted at a lower temperature, 18ºC.

The total mass of contaminants removed from the soil in this temperature settings, can be found in Table 4.10. In general, at 18 ºC, degradation values were between 0 and 95% in the Biotic experiment and between 2 and 95% in EK-Biotic (Table 4.10). After 4 days, in Biotic-18 experiment the highest degradation was achieved by E2, followed by BPA >SMX> IBU> EE2 >CAF ≈TCS ≈DCF ≈OXY ≈CBZ. The EK contributed significantly to the decrease of certain compounds concentration in soil (SMX, EE2, OXY, TCS, DCF and CAF). In EK-Biotic-18 assay, SMX, EE2 and OXY had approximately 15% more degradation, and CAF reached nearly 25% more.

Table 4.10 Degradation of PPCPs in the Biotic conditions with and without EK application at 24 and 18 ºC (cells in light yellow reflect the highest value achieved for each contaminant, considering a minimum 5% difference).

RT (ºC)	Exp	Degradation (%)									
		E2	SMX	BPA	IBU	EE2	OXY	TCS	DCF	CAF	CBZ
24	Biotic-24	90	36	29	25	13	5	0	3	1	0
	EK-Biotic-24	90	51	46	19	21	9	17	16	16	4
18	Biotic-18	95	45	51	16	9	0	0	0	2	0
	Ek-Biotic-18	95	65	50	12	19	15	7	10	29	2

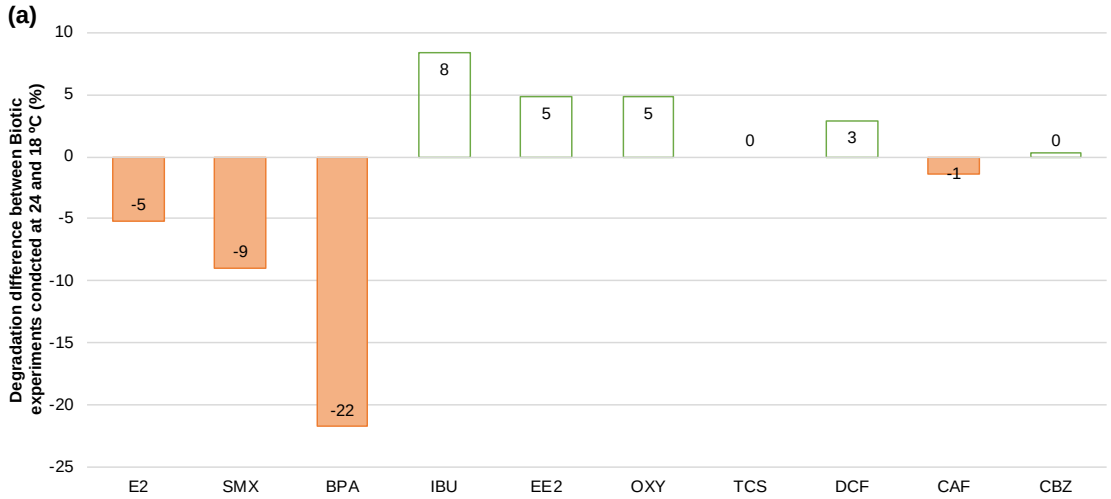
The results show that in a scenario where no EK is applied, the degradation seems to be more efficient in a warmer soil, on the other hand, when EK is applied the contaminants removal seems to work better in the presence of moist soil (Figure 4.8). However, this is not a behaviour displayed by all the contaminants under study, CAF, SMX, TCS, BPA and E2 reached higher levels of removal in lower temperatures as is shown in Figure 4.8. SMX and TCS are better degraded in lower levels of soil moisture.

For E2, the 18ºC allowed to slightly increase E2 degradation ca. 5% both in the Biotic as in the EK-Biotic (Figure 4.8), although no statistical differences were observed (p>0.05). SMX and BPA seem to benefit from the temperature of 18 ºC, being this more significant for BPA with 22 % more degradation (p>0.05) in the Biotic microcosm (Figure 4.8.a). Still, this influence is less pronounced when EK is applied for BPA (only increased +4) whereas the impact on SMX was more pronounced, +14% (Figure 4.8 b).

The rise of temperature also seemed to help reduce IBU in assay Biotic at 24ºC, compared to its equivalent at 18ºC by 9% with the same trend being observed in the EK settings (Figure 4.8). At 18°C, EK-Biotic degradation of EE2 reached 10% more degradation than compared to same conditions without DC (Table 4.10).

For OXY, temperature seemed to exercise opposite influences with and without EK. The Biotic experiment at 18ºC decreased OXY degradation in 8% (p<0.05) (Figure 4.8 a). However, in the presence of EK the degradation of OXY was improved in 6% (Figure 4.8 b).

For TCS, is difficult to deduce the best settings since multiple comparison test only presented significant differences, of 10% between experiments EK-Biotic at 18ºC and at 24ºC, denotating that higher temperatures are more effective (Figure 4.8).



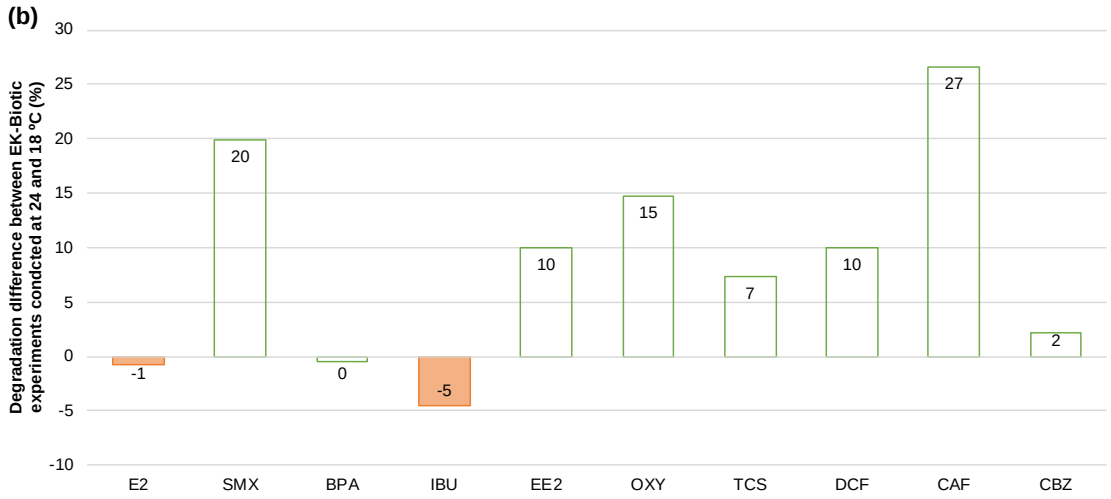


Figure 4.8 Difference between experiments conducted at 18 and 24°C in the Biotic (a) and in the EK-Biotic (b) microcosms (green bars show a positive impact of the 18°C comparing to the 24°C, whereas the orange show a negative impact).

At 18°C DCF is one of the contaminants under study that is least degraded, consistently placing as one of the most recalcitrant three, with a low of 0 ± 3% in Biotic-18 (Table 4.6). There is a positive impact on DCF degradation in the warmer temperature of 24°C of the EK-Biotic experiment, more 6% (p>0.05) (Figure 4.8 b).

The setup that proved to be the most efficient in removing CAF is EK-Biotic at 18°C, in which it achieved a degradation of 29 ± 8% (Table 4.10, Figure 4.8 b). Results also show a mean statistical difference of -27% between Biotic-18 and EK-Biotic-18, proving that CAF removal is more effective when a DC is applied. The EK treatment performed better at lower temperatures since EK-Biotic showed 13% more efficiency at 18ºC than at 24ºC (Figure 4.8 b).

Even at 18ºC, CBZ is still very poorly degraded further supporting the conclusion that it is a recalcitrant PPCP in soil. Still, the higher temperature, 24°C, appears to be the most effective in degrading this type of compound (p>0.05), with an average 3% more CBZ degraded (Table 4.10).

4.4.2. Removal behaviour of each PPCP

4.4.2.1. 17β-Estradiol (E2)

Results show that E2 was the compound most susceptible to degradation in the studied conditions, with an average of 69 ± 2%. In the sterile soil were as follows: Abiotic-24 (19%) < EK-Abiotic-24-W (30%) ≈ EK-Abiotic-24 (33%) (Table 4.6). The application of EK resulted in increases in the overall removal with statistical differences (p<0.05) compared to the experiment without current (Abiotic-24, Table 4.6). Higher degradations of E2 were obtained presence of living organisms, since the three assays with microorganisms (non-sterile soil, biotic conditions) were the ones that displayed higher levels of degradation, average of 94 ±1%, about 70% more

that in the sterile soil (Table 4.6). The complete degradation of E2, 100% (final concentration in soil below LD), was obtained in EK-Biotic-24-W, being this value statistically different from the EK-Biotic were no irrigation was performed (p>0.05). These results show that EK enhanced bioremediation is very effective technology for this PPCP remediation.

4.4.2.2. Sulfamethoxazole (SMX)

The highest level of SMX degradation attained was 81 ± 1% in a setting with electric current and no microorganisms (EK-Abiotic-24-W), whereas the lowest, 14 ± 3% was recorded in a soil that also had no microorganisms. However, without irrigation and DC (Abiotic-24), only 14% of the SMX was removed. In general, all the assays with EK resulted in higher degradation values.

Has held before, it is possible to attain higher degradation values with the synergy of biodegradation, degradation results increased 22% in the Biotic-24 compared to the Abiotic-24, and 8% in EK-Biotic-24 compared to EK-Abiotic-24. However, when the system was irrigated the same tendency was not observed, as EK-Biotic-24-W degraded 32% less SMX that EK-Abiotic-24-W (p<0.05). A possible explanation for these results, is the existing difference of water content between these assays. EK-Abiotic-24-W has approximately 19% water content, whilst EK-Biotic-24-W only reaches 5%. Water content is very important to promote electro-degradation, influencing the process efficiency even without the presence of microorganisms. In a study executed by Liu *et al.,* (2010) the half-life of SMX was 2 days in non-sterile soil under aerobic conditions. Under anoxic conditions, half-life in non-sterile soil was 7 days, showing that SMX dissipated more rapidly in non-sterile soil than in sterile soil. These authors then concluded that biodegradation played a major role in the dissipation of SMX in the soil (Liu *et al.,* 2010).

4.4.2.3. Bisphenol A (BPA)

Removal of BPA increased 17% and 19% when a DC was applied in the biotic and abiotic conditions, respectively (Table 4.6, EK-Biotic-24 and EK-Abiotic-24). The presence of soil's biota improved BPA degradation by 19%, analogous values were achieved in assay EK-Biotic-24, revealing 17% more in comparison to EK-Abiotic-24. The assay that revealed to be the most suitable to degrade BPA was EK-Biotic-24-W with 67 ± 3%, whilst Abiotic losses were only 10 ± 4%, being this the experiment that showed the lowest values of remediation.

Similarly, to previous results, the increased water content contributed positively to EK enhanced bioremediation of BPA, EK-Biotic-24-W increased degradation by 21% through irrigation.

4.4.2.4. Ibuprofen (IBU)

Without the application of EK, higher degradation was found in the Biotic experiment comparing to the Abiotic (20% more at 24ºC), suggesting that bioremediation was beneficial to degrading IBU. Applying DC allowed to increase IBUs removal by 14% in EK-Abiotic-24, other assays did not reveal the same behaviour, although said results did not present differences with statistical meaning (p>0.05).

Controlling soil's water content, trough irrigation, allowed to obtain higher removal efficiency in assay EK-Abiotic-24-W and EK-Biotic-24-W. In these conditions the removal increased 18 and 11% respectively. The highest degradation percentage of IBU in this study was 37 ± 3% found in experiment EK-Abiotic-24-W, probably due to the higher soil moisture content (Figure 4.1). This result follows the same trend as the one obtained for SMX.

Similar findings were reported by Hiller & Šebesta, (2017), that concluded that the sorption of IBU in soil was shown to be pH- and temperature- dependent. The effect of soil pH on IBU sorption was more pronounced than that of temperature. The sorption of the test compound in soil decreased with increasing pH from 4.0 up to 8.0 and the same held for the temperature effect, indicating that the sorption of IBU in soil was exothermic

4.4.2.5. 17α-Ethinylestradiol (EE2)

In the presence on soil biota, the synthetic oestrogen, EE2, only degraded 13 ± 4%, whereas in the abiotic conditions the removal was almost null (Table 4.6). This result is contrary to the one obtained for the natural oestrogen, E2 that degraded 85% more in the presence of microorganisms. The higher stability of EE2 against microbial degradation is attributed to the presence of the ethynyl group which is introduced into the molecule for this specific reason (Stumpe *et al.,* 2009).

When EK treatment was applied to the soil, EE2 was removed more efficiently. Under DC EK-Biotic-24 had 8% more and EK-Abiotic-24 17% more when compared to same conditions without DC (Biotic and Abiotic, respectively, all at 24ºC).

Regarding the influence of bioremediation through microorganisms, once more, although with fewer significant differences, it is possible to assume that EE2 is more susceptible to elimination under the effect of biotic in a soil with higher water content, as EK-Biotic-24-W showed 21% more degradation.

4.4.2.6. Oxybenzone (OXY)

Oxybenzone was the second least degraded compound (simultaneously with TCS) (Table 4.6). In average only 9% of OXY was degraded in all experiments, EK-Abiotic-24-W being the most effective 18 ± 2% (closely followed by EK-Biotic-24-W, 17 ± 6%), although Abiotic also

displayed very low levels of degradation 1 ± 4%. The EK treatment proved to be beneficial to the elimination rates, when applied in Abiotic settings EK improved OXY removal by 8%. Also, Biotic-24 reached 4% higher values under EK, even though without statistical significance (p>0.05).

Throughout the experiments, no major influence from living organisms was detected, since the difference between Biotic and Abiotic was only 4%, at 24ºC (Table 4.6), and among EK-Biotic-24 and EK-Abiotic-24 was 0%, neither displaying significant difference (p>0.05).

Water content seemed to employ some influence, since it affected the degradation by approximately 10%. As similarly to what happen in the previous compounds, watering seems to improve the degradation rate, in both biotic and abiotic scenario, which resulted in more efficient electro-degradation processes.

4.4.2.7. Triclosan (TCS)

As formerly held, TCS proved to be a compound difficult to eliminate from the soil in the here studied settings. The highest removal efficiency was detected in EK-Abiotic-24 with a percentage of 27 ± 4%. Contrarily, Biotic-24 and EK-Biotic-24-W, in which the lowest of 0 ± 4% was registered.

Once more EK is responsible for higher levels of degradation, in assays with living organisms like the natural attenuation control (Biotic-24, Table 4.6), but also in the Abiotic-24 system where it was registered an improve of 22 and 20% in the elimination rate. Unlike before, microcosms with living organisms caused the remediation of TCS to be less effective, since in Biotic-24 the removal was 12% lower than in Abiotic-24 (Table 4.6). Equivalent values were found in experiment EK-Biotic-24 that had 10% less degradation than the equivalent assay with biota, and EK-Biotic-24-W that decreased the remediation by 25% when compared to EK-Abiotic-24-W.

About soil's humidity influence on TCS removal, it is difficult to deduce the best settings since multiple comparison test only presented significant differences of 17% between EK-Biotic-24 vs. EK-Biotic-24-W, denotating that higher humidity content only seems to hinder TCS degradation in a non-sterile setting.

4.4.2.8. Diclofenac (DCF)

Similarly, to what happened to the previous compounds this PPCP was easier degraded when exposed to DC, showing a removal efficiency increase of 9 and 13% (p<0.05) in comparison to Abiotic and Biotic set ups at 24ºC (Table 4.6). The highest degradation, 28 ± 6%, is reached in the EK-Abiotic-24-W set up in which DCF is the third most degraded compound, although the rates in this assay have revealed to be relatively constant. The difference of 2% in DCF degradation between Biotic and Abiotic conditions (Table 4.9), but also of EK-Abiotic-24 and EK-Biotic-24, implies that biota is not very substantial for the elimination of this compound, however

these are non-significant differences(p>0.05). Somewhat contrary results were found in the study of Al-Rajab *et al.,* (2010) which showed that in heat-sterilized soils the dissipation of DCF is much slower, indicating that dissipation is mostly due to biodegradation. Also, Tran *et al.,* (2009) noted that DCF is moderately biodegradable in experiments that used enriched nitrifying activated sludge.

Concerning water content, their effect is harder to assess, since Tukey's multiple comparisons test, did not show a significant difference (p>0.05), except in EK-Abiotic-24, where watering increased the degradation value by 14%. In agreement with what was previously described, a non-significant rate growth of 7% (p>0.05) was registered in the irrigated EK-Biotic-24 experiment.

4.4.2.9. Caffeine (CAF)

After 4 days, abiotic and biotic losses of CAF were negligent showing that this PPCP is recalcitrant in the soil (Table 4.6). The application of EK promoted the degradation of CAF in 21% in sterile conditions (EK-Abiotic-24) and 16% in non-sterile conditions (EK-Biotic-24), being these removals statistically significant from each respective control (p<0.05). This shows that CAF degradation is mainly due to electro-degradation processes and not to biodegradation.

No statistical differences (p>0.05) were found among the experiments in which EK was used, even in the irrigated microcosms. Thus, in the here tested conditions and in 4 days, CAF removal was not affected by the soil microbial community nor by the irrigation.

4.4.2.10. Carbamazepine (CBZ)

Carbamazepine has been found to be highly persistent in the environment (Calisto & Esteves, 2012), and in the present study was the least susceptible compound to degradation, with an elimination average of 4%, only reaching 14 ± 3% in two sets of experiments: EK-Biotic-24-W and EK-Abiotic-24-W, and a low of 0 ± 2% in two assays: Biotic, Abiotic.

Tukey's multiple comparisons test found few significant differences amongst all the assays to which CBZ was subjected. Nevertheless, is possible to deduce based on the results, that soils with higher water content seem to be beneficial for the electro-degradation process. EK-Biotic-24-W showed 10% more degradation than EK-Biotic-24, similarly to EK-Abiotic-24-W which had 8% more than EK-Abiotic-24 (p<0.05).

4.5. PPCPs spatial distribution

The different layers of the cell showed different degradation values. Depth and the soil layers influence by either the anode or cathode, are some of the characteristics that affect removal rates, since these can vary with the amount of water present in the soil, the type of compound to

be degraded, temperature and the value of the applied electric current. The results obtained in this study are displayed in Figure 4.9.

The presence of these compounds occurred mainly in the first level, C1 and A1, and punctually in the second level, C2 and A2. None of the contaminants were detected at the deepest level of the cell, corresponding to C3 and A3, suggesting that there was no deep infiltration by these PPCPs. This is explained by the way the soil spiking was carried out, that is, the contaminants were essentially placed on the surface of the cell, through irrigation with spiked water at time zero.

No major conclusions were drawn on PPCPs mobilization towards the cathode or anode as the differences found between both compartments were mainly attributed to an inhomogeneous spiking procedure (manual irrigation with spiked deionised water). Still, a more detailed analysis was performed *per* compound in only two experiments: Biotic-18 and EK-Biotic-18. These experiments were chosen because: (i) no daily irrigation was performed (thus minimizing the errors added by an inhomogeneous irrigation) and (ii) the soil temperature is more realistic.

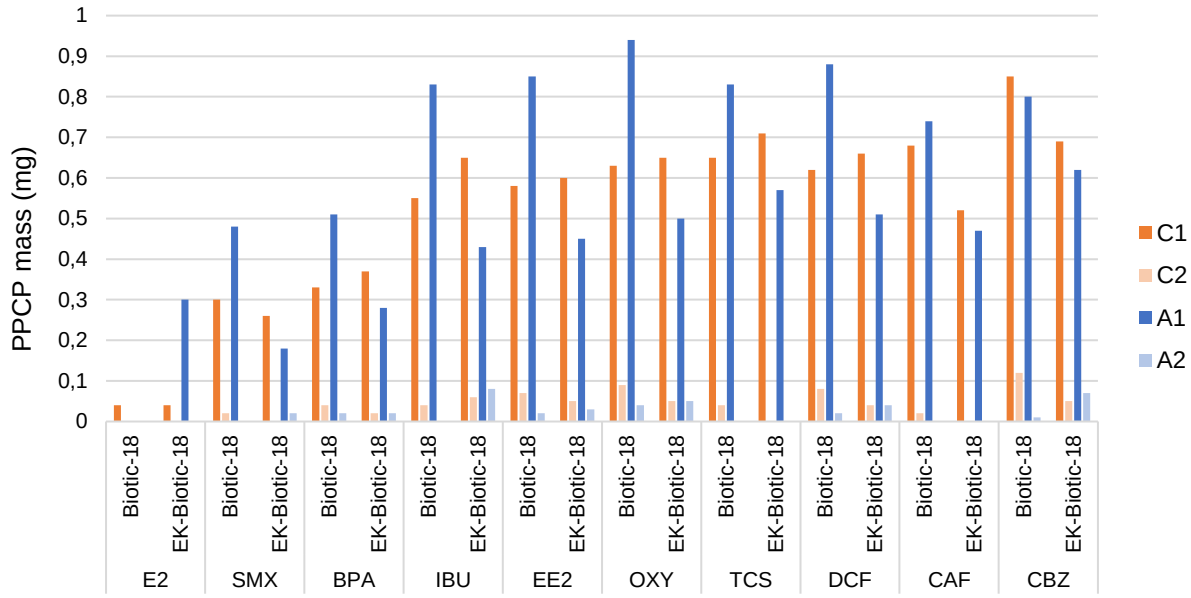


Figure 4.9 Spatial distribution of the studied compounds in experiment Biotic-18 and EK-Biotic-18, through the two top levels of the cell (C1, C2 and A1, A2). None of the contaminants were found in C3 and A3.

4.5.1. 17β-Estradiol (E2)

When studying the results of Biotic-18 obtained in C2 an C3 sections, the values for the deepest sections of the cathode side, showed no presence of the contaminant, and in C1 only 0.03 mg were detected. Section A1 presented 0.06 mg of E2 compared, with none being detected in layers A2 and A3, suggesting that E2 is easily retained in the superficial areas of the soil or it

is easily degraded upon arrival to A2 and A3 layers. Regarding EK-Biotic-18 influence on E2 migration no results were found.

4.5.2. Sulfamethoxazole (SMX)

When comparing the different cathode sections in Biotic-18 experience, C1 showed presence of SMX as well as C2 although, with 0.28 mg less than C1 which had 0.43 ± 0.03 mg. Similar results were obtained in the anode side of the cell, with A1 exhibiting 0.39 ± 0.04 mg of SMX in this section. In the deeper sections of the cell, the compound was not detected, suggesting that it did not enter deep in the soil. In EK-Biotic-18, C1 and C2 registered small amounts of SMX, 0.26 ± 0.02 mg and 0.03 ± 0.10 mg respectively, while in section C3 no presence was registered. Regarding the side of the cell under the anode influence, A1 and A2 both registered presence of this contaminant, but at different concentrations, A1 had 0.18 ± 0.04 mg and A2 0.02 ± 0.01 mg. This suggests that either SMX is more easily degraded in the anode section or it migrates towards the cathode side, most probably via electroosmosis, where it is degraded.

4.5.3. Bisphenol A (BPA)

In Biotic-18 assay, BPA's presence was detected in the most superficial sections of the cell, with C1 showing 0.31 ± 0.03 mg and A1 0.29 ± 0.07 mg. Section C2 also exhibited very small levels of this compound with 0.01 ± 0.02 mg. Regarding EK-Biotic-18, the compound was detected in C1 and C2 and A1 and A2. The sections on the second level, C2 and A2, displayed very small amounts of BPA with 0.04 ± 0.0 mg and 0.01 ± 0.0 mg, whilst the first level of the cell, C1 and A1, exhibited higher amounts of this compound with 0.32 ± 0.01 mg in C1 and 0.26 ± 0.01 mg in A1. BPA was not present in the deeper sections C3 and A3 in both experiments. Similarly, to SMX, these results suggest that either BPA is more easily degraded in the anode section or it migrates towards the cathode side, most probably via electroosmosis, where it is degraded.

4.5.4. Ibuprofen (IBU)

In Biotic-18 test IBU was not detected in the deeper sections of the cell (C2, C3, A2 and A3), in A1 registered the highest value of 0.61 ± 0.09 mg and 0.49 ± 0.04 mg in C1. In EK-Biotic-18, like the previous results, IBU was present in the first two section levels, though in the second level (C2 and A2) presented much fewer amounts, with a difference of 0.59 mg and 0.35 mg less, between their adjacent surface sections, which displayed 0.65 ± 0.02 mg and 0.43 ± 0.05 mg, correspondingly. Contrary to SMX and BPA, this compound is more concentrated in the anode side than in the cathode side.

4.5.5. 17α-Ethinylestradiol (EE2)

In both studied tests, Biotic-18 and EK-Biotic-18, EE2 was found in the first two levels of the cell, and as before, C2 and A2 showed much smaller quantities, almost inexistent. For Biotic-18, 0.07 ± 0.03 mg and 0.02 ± 0.03 mg where the corresponding values detected in C2 and A2. C1 and A1 reached higher values, with A1 being the highest with 0.85 ± 0.20 mg, and C1 having 0.28 mg less than the latter. In EK-Biotic-18 the maximum quantities were found in C1, 0.60 ± 0.07 mg, followed by A1, 0.45 ± 0.00 mg. The values detected in C2 and A2 were very below the previous, with 0.05 and 0.03 ± 0.01 mg, respectively. No presence of EE2 was found in the lowest level of the cell, C3 and A3, in both experiments.

4.5.6. Oxybenzone (OXY)

Oxy was detected in Biotic-18 and EK-Biotic-18, with more persistence in the first setting addressed. The values registered in the sections were cathode was inserted, were quite similar, in Biotic-18 C1 had 0.63 ± 0.19 mg and C2 0.07 ± 0.03 mg. Equally in EK-Biotic-18, C1 displayed 0.65 ± 0.06 mg and C2 0.05 ± 0.01 mg. In the sections most affected by anode, in the A1 section of Biotic-18 the value of 0.94 ± 0.20 mg was the highest detected, followed by A1 in EK-Biotic-18 0.50 ± 0.03 mg. In both assays, section A2, displayed the same value for OXY, 0.05 ± 0.02 mg. Just like before, no OXY was present in the deeper sections of both trials.

4.5.6.1 Triclosan (TCS)

In Biotic-18, TCS could be found in C1 and C2, but with significantly different values, and also in A1. Similarly, to the before analysed compounds, the surface sections of C1 and A1 presented the higher values, with 0.65 ± 0.18 mg and 0.82 ± 0.13 mg, respectively. In the second level of the cell, no TCS was found in A2, but it was traceable in C2 with 0.04 ± 0.10 mg.

Regarding EK-Biotic-18, TCS was only detected in C1 and A1, with the first one showing 0.14 mg more than A1 that displayed 0.57 ± 0.05 mg.

4.5.6.2. Diclofenac (DCF)

Equivalently to what occurred with OXY, the values registered in the sections were cathode was inserted, were quite close, in both experiments. Section C1 in Biotic-18 had 0.62 ± 0.20 mg and C2 0.08 ± 0.01 mg, while EK-Biotic-18 had 0.66 ± 0.04 mg in C1 and 0.04 ± 0.01 mg in C2. The values in the sections affected by the anode, were greater in the shallowest sections of both experiments, although a traceable amount of 0.04 ± 0.01 mg was detected in A2 of EK-Biotic-18, and 0.02 ± 0.04 mg in the same section of Biotic-18. The value of 0.88 ± 0.22 mg was the highest detected, and it was registered in A1 of Biotic-18, the matching section in EK-

Biotic-18 registered a presence of DCF in the order of 0.51 ± 0.00 mg. No presence of DCF was detected in the deeper sections of both assays.

4.5.6.3. Caffeine (CAF)

CAF seemed to be more degradable under the influence of EK process, and for that reason Biotic-18 showed higher values for this compound than its contender EK-Biotic-18. This contaminant was mostly detected in C1 and A1, but it could be found in residual amounts, 0.02 ± 0.05 mg, in section C2 of Biotic-18 assay. The highest value was registered was 0.74 ± 0.35 mg in A1 of Biotic-18, followed by section C1 of the same experiment, 0.68 ± 0.08 mg. In EK-Biotic-18 CAF could only be found in C1 and A1, with 0.52 ± 0.04 mg and 0.47 ± 0.12 mg, correspondingly.

4.5.6.4. Carbamazepine (CBZ)

As one of the most recalcitrant PPCPs here studied, CBZ was found in both experiments in the first two levels. Just like CAF, this compound was more easily removed when on the effect of EK treatment, and so it was more present in the sections of Biotic-18. In sections C1 and A1 the compound existed at higher values, and in Biotic-18 reached 0.85 ± 0.14 mg and 0.80 ± 0.43 mg, respectively. It was also possible to detect it in the second level of the cell, with C2 being more likely to display it since it had 0.11 ± 0.02 mg more than A2 with 0.01 ± 0.02 mg. Regarding EK-Biotic-18, similar measures were found, with the bigger amounts being registered in C1 and A1, with the values of 0.69 ± 0.05 mg and 0.62 ± 0.04 mg respectively, followed by residual values of 0.05 ± 0.01 mg and 0.07 ± 0.02 mg in C2 and A2.

5. Conclusion

The main objective of this dissertation was to study the electrokinetic remediation of soils spiked with a mixture of ten common PPCPs, in different environmental conditions, aiming to decrease the risk of organic contaminants uptake by crops. The said PPCPs were CAF, SMX, CBZ, DCF, OXY, TCS, BPA, E2, EE2 and IBU. Overall, the contaminant more susceptible to degradation was E2 (<LD) followed by BPA and SMX that reached 60% when EK was applied in non-sterile conditions. On the other hand, the compounds more recalcitrant to degradation in the studied conditions were CBZ, OXY with an overall maximum degradation of 20%.

EK process showed to be a reliable choice for the removal of these organic compounds, showing an improvement of approximately 10% in degradation rates in comparison to the assays with similar characteristics but without EK, in only 4 days.

Regarding the different removal mechanisms, the contribution of soils microbiota proved to be a significant factor for the degradation of several PPCPs; in sterile soil 6% of the total mass load of PPCPs was removed whereas the Biotic removals achieved 20%, which means that ca.14% of the achieved degradation is due to biodegradation processes.

No major temperature variations were observed in the soil, except in experiment EK-Abiotic-24 and EK-Abiotic-24-W, where soil temperature increased more than 3°C, which may be related to the ohmic heating generated in the system by the electrical resistance of electrodes.

Electroheating may be used as an advantage since the uniform temperature may allow a uniform removal of the contaminants and a more efficient use of the energy. Concerning water content in the soil, the lowest values were registered in the shallower surfaces of the cells, more exposed to evaporation and to the heat generated during the electrokinetic remediation.

The results show that in a scenario where no EK is applied, the degradation seems to be more efficient in a cooler soil; on the other hand, when EK is applied the contaminants, removal seems to work better in the presence of lower temperatures and moist soil. However, this is not a behaviour displayed by all the contaminants under study, as CAF, SMX, TCS, BPA and E2 reached higher levels of removal in lower temperatures. SMX and TCS are better degraded in lower levels of soil moisture.

It is important to understand that these results are directly related to the physicochemical properties of simultaneously the soils and the contaminants studied, so if another soil with different properties or another contaminant is used, it is imperative to understand its characteristics and choose the most suitable remediation technique.

Still, in this study EK showed to be a promising *in situ* technology for CECs removal from soil, consequently decreasing human and environmental associated risks, and thus reducing the chance of organic contaminants uptake by crops.

6. Future developments

The results achieved in this research support EK as an important remediation option for soil contaminated with PCPPs, although further studies and process optimization should be made.

In order to achieve quality remediation of contaminated soil it is important to understand the type of soil and physiochemical characteristics associated with it, and conduct a contamination inventory as accurate as possible, that is, to determine the state and reason of soil contamination. With that in mind, studying how the compound mobilization behaves in the environment, when subjected to severe temperature changes, different water conditions and sunlight is also of significant importance. To achieve it, long-term tests with soils subjected to real environmental conditions in relevant sites should be promoted.

Changes in the experimental design, such as other electrodes to promote better PPCPs degradation, or natural enhancing agents and techniques, should be tested to improve the efficiency of EK remediation.

In the present study, soil samples were collected and frozen immediately after the end of each assay to enable further analysis on the evolution of the microbiological community (bacteria and fungi) present in the soil. These samples may also be used to ascertain which physicochemical and biological changes may have occurred after EK process, like changes in infiltration rate, redox status, salinity and respiration rate.

Added care must be given to the comprehension of the degradation process of this type of compounds, to assess the formation of possible by-products and their characteristics, as well as their effect on soil, which are still relatively unknown.

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ANNEXES

5.2 Special hazards arising from the substance or mixture
No data available

5.3 Advice for firefighters
Wear self-contained breathing apparatus for firefighting if necessary.

5.4 Further information
No data available

SECTION 6: Accidental release measures

6.1 Personal precautions, protective equipment and emergency procedures
Use personal protective equipment. Avoid dust formation. Avoid breathing vapours, mist or gas. Ensure adequate ventilation. Avoid breathing dust.
For personal protection see section 8.

6.2 Environmental precautions
Prevent further leakage or spillage if safe to do so. Do not let product enter drains. Discharge into the environment must be avoided.

6.3 Methods and materials for containment and cleaning up
Pick up and arrange disposal without creating dust. Sweep up and shovel. Keep in suitable, closed containers for disposal.

6.4 Reference to other sections
For disposal see section 13.

SECTION 7: Handling and storage

7.1 Precautions for safe handling
Avoid contact with skin and eyes. Avoid formation of dust and aerosols.
Provide appropriate exhaust ventilation at places where dust is formed.
For precautions see section 2.2.

7.2 Conditions for safe storage, including any incompatibilities
Keep container tightly closed in a dry and well-ventilated place. Store in cool place.

7.3 Specific end use(s)
Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

SECTION 8: Exposure controls/personal protection

8.1 Control parameters

Components with workplace control parameters
Contains no substances with occupational exposure limit values.

8.2 Exposure controls

Appropriate engineering controls
Handle in accordance with good industrial hygiene and safety practice. Wash hands before breaks and at the end of workday.

Personal protective equipment

Eye/face protection
Safety glasses with side-shields conforming to EN166 Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

Skin protection
Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

The selected protective gloves have to satisfy the specifications of EU Directive 89/686/EEC and the standard EN 374 derived from it.

SAFETY DATA SHEET

according to Regulation (EC) No. 1907/2006
Version 5.0 Revision Date 07.09.2018
Print Date 25.03.2019

SECTION 1: Identification of the substance/mixture and of the company/undertaking

1.1 Product identifiers

Product name	:	Caffeine
Product Number	:	PHR1009
Brand	:	Sigma-Aldrich
REACH No.	:	A registration number is not available for this substance as the substance or its uses are exempted from registration, the annual tonnage does not require a registration or the registration is envisaged for a later registration deadline.
CAS-No.	:	58-08-2

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses	:	Laboratory chemicals, Manufacture of substances

1.3 Details of the supplier of the safety data sheet

Company	:	Sigma-Aldrich Company Ltd. The Old Brickyard NEW ROAD, GILLINGHAM Dorset SP8 4XT UNITED KINGDOM
Telephone	:	+44 (0)1747 833000
Fax	:	+44 (0)1747 833313
E-mail address	:	eurtechserv@sial.com

1.4 Emergency telephone number

Emergency Phone #		+44 (0)870 8200418 (CHEMTREC)

SECTION 2: Hazards identification

2.1 Classification of the substance or mixture

Classification according to Regulation (EC) No 1272/2008
Acute toxicity, Oral (Category 4), H302

For the full text of the H-Statements mentioned in this Section, see Section 16.

2.2 Label elements

Labelling according Regulation (EC) No 1272/2008
Pictogram

Signal word Warning

Hazard statement(s)
H302 Harmful if swallowed.

Precautionary statement(s)
P264 Wash skin thoroughly after handling.

Specific target organ toxicity - repeated exposure
No data available

Aspiration hazard
No data available

Additional Information
RTECS: Not available

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

2.3 After absorption of toxic quantities:
Diarrhoea, Vomiting, agitation, restlessness, Headache
Systemic effects:
drop in blood pressure, tachycardia, collapse
Handle in accordance with good industrial hygiene and safety practice.

SEC Liver - Irregularities - Based on Human Evidence

3.1

SECTION 12: Ecological information

12.1 Toxicity

Toxicity to fish	LC50 - Leuciscus idus (Golden orfe) - ca. 87 mg/l - 96 h (DIN 38412 part 15)
	NOEC - Leuciscus idus (Golden orfe) - 46 mg/l - 96 h (DIN 38412 part 15)
Toxicity to daphnia and other aquatic invertebrates	static test EC50 - Daphnia magna (Water flea) - 182 mg/l - 48 h (DIN 38412)
Toxicity to algae	EC50 - Desmodesmus subspicatus (green algae) - > 100 mg/l - 72 h (OECD Test Guideline 201)
	NOEC - Desmodesmus subspicatus (green algae) - 6.25 mg/l - 72 h (OECD Test Guideline 201)
Toxicity to bacteria	BRINGMANN-KüHN-TEST EC50 - Pseudomonas putida - 3,490 mg/l - 17 h Remarks: (ECHA)
	EC50 - activated sludge - > 1,000 mg/l - 3 h (OECD Test Guideline 209)

SEC

4.1

12.2 Persistence and degradability
Biodegradability: Result: 90 - 100 % - Readily biodegradable.
(OECD Test Guideline 301A)

12.3 Bioaccumulative potential
No data available

12.4 Mobility in soil
No data available

12.5 Results of PBT and vPvB assessment
This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

12.6 Other adverse effects
Harmful to aquatic life.

Discharge into the environment must be avoided.

SAFETY DATA SHEET

according to Regulation (EC) No. 1907/2006
Version 6.6 Revision Date 28.09.2017
Print Date 10.11.2018

ECTION 1: Identification of the substance/mixture and of the company/undertaking

.1 Product identifiers

Product name	:	Acetonitrile
Product Number	:	271004
Brand	:	Sigma-Aldrich
Index-No.	:	608-001-00-3
REACH No.	:	01-2119471307-38-XXXX
CAS-No.	:	75-05-8

.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses	:	Laboratory chemicals, Manufacture of substances

.3 Details of the supplier of the safety data sheet

Company	:	Sigma-Aldrich Company Ltd. The Old Brickyard NEW ROAD, GILLINGHAM Dorset SP8 4XT UNITED KINGDOM
Telephone	:	+44 (0)1747 833000
Fax	:	+44 (0)1747 833313
E-mail address	:	eurtechserv@sial.com

.4 Emergency telephone number

Emergency Phone # +44 (0)870 8200418 (CHEMTREC)

ECTION 2: Hazards identification

.1 Classification of the substance or mixture

Classification according to Regulation (EC) No 1272/2008
Flammable liquids (Category 2), H225
Acute toxicity, Oral (Category 4), H302
Acute toxicity, Inhalation (Category 4), H332
Acute toxicity, Dermal (Category 4), H312
Eye irritation (Category 2), H319

For the full text of the H-Statements mentioned in this Section, see Section 16.

.2 Label elements

Labelling according Regulation (EC) No 1272/2008
Pictogram

Signal word Danger

Hazard statement(s)
H225 Highly flammable liquid and vapour.
H302 + H312 + H332 Harmful if swallowed, in contact with skin or if inhaled.

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SAFETY DATA SHEET

according to Regulation (EC) No. 1907/2006
Version 5.8 Revision Date 13.07.2017
Print Date 10.11.2018

SECTION 1: Identification of the substance/mixture and of the company/undertaking

1.1 Product identifiers

Product name	:	Bisphenol A
Product Number	:	239658
Brand	:	Aldrich
Index-No.	:	604-030-00-0
REACH No.	:	A registration number is not available for this substance as the substance or its uses are exempted from registration, the annual tonnage does not require a registration or the registration is envisaged for a later registration deadline.
CAS-No.	:	80-05-7

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Manufacture of substances

1.3 Details of the supplier of the safety data sheet

Company : Sigma-Aldrich Company Ltd.
The Old Brickyard
NEW ROAD, GILLINGHAM
Dorset
SP8 4XT
UNITED KINGDOM

Telephone : +44 (0)1747 833000
Fax : +44 (0)1747 833313
E-mail address : eurtechserv@sial.com

1.4 Emergency telephone number

Emergency Phone # +44 (0)870 8200418 (CHEMTREC)

SECTION 2: Hazards identification

2.1 Classification of the substance or mixture

Classification according to Regulation (EC) No 1272/2008
Serious eye damage (Category 1), H318
Skin sensitisation (Category 1), H317
Reproductive toxicity (Category 2), H361f
Specific target organ toxicity - single exposure (Category 3), Respiratory system, H335
Chronic aquatic toxicity (Category 2), H411

For the full text of the H-Statements mentioned in this Section, see Section 16.

2.2 Label elements

Labelling according Regulation (EC) No 1272/2008
Pictogram

Signal word Danger

Hazard statement(s)

H317	May cause an allergic skin reaction.
H318	Causes serious eye damage.
H335	May cause respiratory irritation.
H361f	Suspected of damaging fertility.
H411	Toxic to aquatic life with long lasting effects.

Precautionary statement(s)

P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.
P305 + P351 + P338 + P310	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/doctor.
Supplemental Hazard Statements	none

2.3 Other hazards

This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

SECTION 3: Composition/information on ingredients

3.1 Substances

Synonyms	:	2,2-Bis(4-hydroxyphenyl)propane4,4'-Isopropylidenediphenol
Formula	:	$C_{15}H_{16}O_2$
Molecular weight	:	228.29 g/mol
CAS-No.	:	80-05-7
EC-No.	:	201-245-8
Index-No.	:	604-030-00-0

Hazardous ingredients according to Regulation (EC) No 1272/2008

Component		Classification	Concentration
Bisphenol A Included in the Candidate List of Substances of Very High Concern (SVHC) according to Regulation (EC) No. 1907/2006 (REACH)			
CAS-No. EC-No. Index-No.	80-05-7 201-245-8 604-030-00-0	Eye Dam. 1; Skin Sens. 1; Repr. 2; STOT SE 3; Aquatic Chronic 2; H318, H317, H361f, H335, H411	<= 100 %

For the full text of the H-Statements mentioned in this Section, see Section 16.

SECTION 4: First aid measures

4.1 Description of first aid measures

General advice
Consult a physician. Show this safety data sheet to the doctor in attendance.

If inhaled
If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

In case of skin contact
Wash off with soap and plenty of water. Consult a physician.

In case of eye contact
Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.

If swallowed
Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

SIGMA-ALDRICH

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SAFETY DATA SHEET

according to Regulation (EC) No. 1907/2006
Version 5.2 Revision Date 28.10.2013
Print Date 25.03.2019

SECTION 1: Identification of the substance/mixture and of the company/undertaking

1.1 Product identifiers

Product name	:	Carbamazepine
Product Number	:	94496
Brand	:	Sigma-Aldrich
REACH No.	:	A registration number is not available for this substance as the substance or its uses are exempted from registration, the annual tonnage does not require a registration or the registration is envisaged for a later registration deadline.
CAS-No.	:	298-46-4

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses	:	Laboratory chemicals, Manufacture of substances

1.3 Details of the supplier of the safety data sheet

Company	:	Sigma-Aldrich Company Ltd. The Old Brickyard NEW ROAD, GILLINGHAM Dorset SP8 4XT UNITED KINGDOM
Telephone	:	+44 (0)1747 833000
Fax	:	+44 (0)1747 833313
E-mail address	:	eurtechserv@sial.com

1.4 Emergency telephone number

Emergency Phone #	:	+44 (0)870 8200418 (CHEMTREC)

SECTION 2: Hazards identification

2.1 Classification of the substance or mixture

Classification according to Regulation (EC) No 1272/2008
Acute toxicity, Oral (Category 4), H302
Respiratory sensitisation (Category 1), H334
Skin sensitisation (Category 1), H317

For the full text of the H-Statements mentioned in this Section, see Section 16.

Classification according to EU Directives 67/548/EEC or 1999/45/EC
Xn Harmful R22, R42/43

For the full text of the R-phrases mentioned in this Section, see Section 16.

2.2 Label elements

Labelling according Regulation (EC) No 1272/2008
Pictogram

Signal word Danger

Hazard statement(s)
H302 Harmful if swallowed.
H317 May cause an allergic skin reaction.

H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.

Precautionary statement(s)

P261	Avoid breathing dust.
P280	Wear protective gloves.
P342 + P311	If experiencing respiratory symptoms: Call a POISON CENTER or doctor/ physician.
Supplemental Hazard Statements	none

2.3 Other hazards - none

SECTION 3: Composition/information on ingredients

3.1 Substances

Formula	:	C15H12N2O
Molecular Weight	:	236.27 g/mol
CAS-No.	:	298-46-4
EC-No.	:	206-062-7

Hazardous ingredients according to Regulation (EC) No 1272/2008

Component		Classification	Concentration
Carbamazepine			
CAS-No. EC-No.	298-46-4 206-062-7	Acute Tox. 4; Resp. Sens. 1; Skin Sens. 1; H302, H317, H334	<= 100 %

Hazardous ingredients according to Directive 1999/45/EC

Component		Classification	Concentration
Carbamazepine			
CAS-No. EC-No.	298-46-4 206-062-7	Xn, , R22 - R42/43R22 - R42/43	<= 100 %

For the full text of the H-Statements and R-Phrases mentioned in this Section, see Section 16

SECTION 4: First aid measures

4.1 Description of first aid measures

General advice
Consult a physician. Show this safety data sheet to the doctor in attendance.

If inhaled
If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

In case of skin contact
Wash off with soap and plenty of water. Consult a physician.

In case of eye contact
Flush eyes with water as a precaution.

If swallowed
Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

4.2 Most important symptoms and effects, both acute and delayed
The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

4.3 Indication of any immediate medical attention and special treatment needed
no data available

SAFETY DATA SHEET

according to Regulation (EC) No. 453/2010
Version 5.3 Revision Date 12.10.2015
Print Date 25.03.2019

SECTION 1: Identification of the substance/mixture and of the company/undertaking

1.1 Product identifiers

Product name : Diclofenac sodium salt

Product Number : 93484
Brand : Sigma-Aldrich
REACH No. : A registration number is not available for this substance as the substance or its uses are exempted from registration, the annual tonnage does not require a registration or the registration is envisaged for a later registration deadline.
CAS-No. : 15307-79-6

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Manufacture of substances

1.3 Details of the supplier of the safety data sheet

Company : Sigma-Aldrich Company Ltd.
The Old Brickyard
NEW ROAD, GILLINGHAM
Dorset
SP8 4XT
UNITED KINGDOM

Telephone : +44 (0)1747 833000
Fax : +44 (0)1747 833313
E-mail address : eurtechserv@sial.com

1.4 Emergency telephone number

Emergency Phone # +44 (0)870 8200418 (CHEMTREC)

SECTION 2: Hazards identification

2.1 Classification of the substance or mixture

Classification according to Regulation (EC) No 1272/2008
Acute toxicity, Oral (Category 3), H301

For the full text of the H-Statements mentioned in this Section, see Section 16.

2.2 Label elements

Labelling according Regulation (EC) No 1272/2008
Pictogram

Signal word Danger

Hazard statement(s)
H301 Toxic if swallowed.

Precautionary statement(s)
P301 + P310 IF SWALLOWED: Immediately call a POISON CENTER or doctor/

physician.

Supplemental Hazard Statements none

2.3 Other hazards

This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

SECTION 3: Composition/information on ingredients

3.1 Substances

Formula : C14H10Cl2NNaO2
Molecular weight : 318.1 g/mol
CAS-No. : 15307-79-6
EC-No. : 239-346-4

Hazardous ingredients according to Regulation (EC) No 1272/2008

Component		Classification	Concentration
Sodium [2-[(2,6-dichlorophenyl)amino]phenyl]acetate			
CAS-No. EC-No.	15307-79-6 239-346-4	Acute Tox. 3; H301	<= 100 %

For the full text of the H-Statements mentioned in this Section, see Section 16.

SECTION 4: First aid measures

4.1 Description of first aid measures

General advice
Consult a physician. Show this safety data sheet to the doctor in attendance.

If inhaled
If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

In case of skin contact
Wash off with soap and plenty of water. Take victim immediately to hospital. Consult a physician.

In case of eye contact
Flush eyes with water as a precaution.

If swallowed
Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

4.2 Most important symptoms and effects, both acute and delayed
The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

4.3 Indication of any immediate medical attention and special treatment needed
No data available

SECTION 5: Firefighting measures

5.1 Extinguishing media

Suitable extinguishing media
Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

5.2 Special hazards arising from the substance or mixture
Carbon oxides, Nitrogen oxides (NOx), Hydrogen chloride gas, Sodium oxides

5.3 Advice for firefighters
Wear self-contained breathing apparatus for firefighting if necessary.

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SAFETY DATA SHEET

according to Regulation (EC) No. 1907/2006
Version 5.2 Revision Date 19.06.2014
Print Date 10.11.2018

SECTION 1: Identification of the substance/mixture and of the company/undertaking

1.1 Product identifiers

Product name : β-Estradiol

Product Number : E8875
Brand : Sigma
REACH No. : A registration number is not available for this substance as the substance or its uses are exempted from registration, the annual tonnage does not require a registration or the registration is envisaged for a later registration deadline.
CAS-No. : 50-28-2

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Manufacture of substances

1.3 Details of the supplier of the safety data sheet

Company : Sigma-Aldrich Company Ltd.
The Old Brickyard
NEW ROAD, GILLINGHAM
Dorset
SP8 4XT
UNITED KINGDOM

Telephone : +44 (0)1747 833000
Fax : +44 (0)1747 833313
E-mail address : eurtechserv@sial.com

1.4 Emergency telephone number

Emergency Phone # : +44 (0)870 8200418 (CHEMTREC)

SECTION 2: Hazards identification

2.1 Classification of the substance or mixture

Classification according to Regulation (EC) No 1272/2008
Carcinogenicity (Category 2), H351
Reproductive toxicity (Category 1A), H360Fd
Effects on or via lactation, H362

For the full text of the H-Statements mentioned in this Section, see Section 16.

Classification according to EU Directives 67/548/EEC or 1999/45/EC
T Toxic R60, R40, R63, R64

For the full text of the R-phrases mentioned in this Section, see Section 16.

2.2 Label elements

Labelling according Regulation (EC) No 1272/2008
Pictogram

Signal word Danger

Hazard statement(s)
H351 Suspected of causing cancer.

H360Fd May damage fertility. Suspected of damaging the unborn child.
H362 May cause harm to breast-fed children.

Precautionary statement(s)
P201 Obtain special instructions before use.
P263 Avoid contact during pregnancy/ while nursing.
P281 Use personal protective equipment as required.
P308 + P313 IF exposed or concerned: Get medical advice/ attention.

Supplemental Hazard Statements none

Restricted to professional users.

2.3 Other hazards - none

SECTION 3: Composition/information on ingredients

3.1 Substances

Synonyms : 3,17β-Dihydroxy-1,3,5(10)-estratriene
1,3,5-Estratriene-3,17β-diol
Dihydrofolliculin
17β-Estradiol

Formula : $C_{18}H_{24}O_2$
Molecular Weight : 272.38 g/mol
CAS-No. : 50-28-2
EC-No. : 200-023-8

Hazardous ingredients according to Regulation (EC) No 1272/2008

Component		Classification	Concentration
Estradiol			
CAS-No. EC-No.	50-28-2 200-023-8	Carc. 2; Repr. 1A; Lact. ; H351, H360Fd, H362	<= 100 %

Hazardous ingredients according to Directive 1999/45/EC

Component		Classification	Concentration
Estradiol			
CAS-No. EC-No.	50-28-2 200-023-8	T, R60 - R40 - R63 - R64	<= 100 %

For the full text of the H-Statements and R-Phrases mentioned in this Section, see Section 16

SECTION 4: First aid measures

4.1 Description of first aid measures

General advice
Consult a physician. Show this safety data sheet to the doctor in attendance.

If inhaled
If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

In case of skin contact
Wash off with soap and plenty of water. Consult a physician.

In case of eye contact
Flush eyes with water as a precaution.

If swallowed
Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

According to European Directive 67/548/EEC as amended.
Hazard symbol(s)

R-phrase(s)

R45	May cause cancer.
R22	Also harmful if swallowed.

S-phrase(s)

S53	Avoid exposure - obtain special instructions before use.
S45	In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).
S36/37/39	Wear suitable protective clothing, gloves and eye/face protection.

Restricted to professional users.

2.3 Other hazards - none

3. COMPOSITION/INFORMATION ON INGREDIENTS

3.1 Substances

Synonyms : 17α-Ethynyl-1,3,5(10)-estratriene-3,17β-diol
19-Nor-1,3,5(10),17α-pregnatrien-20-yne-3,17-diol
Ethinylestradiol

Formula : $C_{20}H_{24}O_2$
Molecular Weight : 296.40 g/mol

Component		Concentration
17α-Ethynylestradiol		
CAS-No.	57-63-6	-
EC-No.	200-342-2	

4. FIRST AID MEASURES

4.1 Description of first aid measures

General advice
Consult a physician. Show this safety data sheet to the doctor in attendance.

If inhaled
If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

In case of skin contact
Wash off with soap and plenty of water. Consult a physician.

In case of eye contact
Flush eyes with water as a precaution.

If swallowed
Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

4.2 Most important symptoms and effects, both acute and delayed

4.3 Indication of any immediate medical attention and special treatment needed
no data available

5. FIREFIGHTING MEASURES

5.1 Extinguishing media

Suitable extinguishing media
Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

SAFETY DATA SHEET

according to Regulation (EC) No. 1907/2006
Version 5.1 Revision Date 13.11.2012
Print Date 10.11.2018

1. IDENTIFICATION OF THE SUBSTANCE/MIXTURE AND OF THE COMPANY/UNDERTAKING

1.1 Product identifiers

Product name : 17α-Ethynylestradiol

Product Number : E4876
Brand : Sigma
CAS-No. : 57-63-6

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Manufacture of substances

1.3 Details of the supplier of the safety data sheet

Company : Sigma-Aldrich Company Ltd.
The Old Brickyard
NEW ROAD, GILLINGHAM
Dorset
SP8 4XT
UNITED KINGDOM

Telephone : +44 (0)1747 833000
Fax : +44 (0)1747 833313
E-mail address : eurtechserv@sial.com

1.4 Emergency telephone number

Emergency Phone # : +44 (0)870 8200418 (CHEMTREC)

2. HAZARDS IDENTIFICATION

2.1 Classification of the substance or mixture

Classification according to Regulation (EC) No 1272/2008 [EU-GHS/CLP]
Acute toxicity, Oral (Category 4)
Carcinogenicity (Category 1B)

Classification according to EU Directives 67/548/EEC or 1999/45/EC
May cause cancer. Harmful if swallowed.

2.2 Label elements

Labelling according Regulation (EC) No 1272/2008 [CLP]
Pictogram

Signal word Danger

Hazard statement(s)

H302	Harmful if swallowed.
H350	May cause cancer.

Precautionary statement(s)

P201	Obtain special instructions before use.
P308 + P313	IF exposed or concerned: Get medical advice/ attention.

Supplemental Hazard Statements none

Restricted to professional users.

P270	Do not eat, drink or smoke when using this product.
P301 + P312 + P330	IF SWALLOWED: Call a POISON CENTER/doctor if you feel unwell. Rinse mouth.
P501	Dispose of contents/ container to an approved waste disposal plant.
Supplemental Hazard Statements	none

2.3 Other hazards

This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

SECTION 3: Composition/information on ingredients

3.1 Substances

Synonyms	:	α-Methyl-4-(isobutyl)phenylacetic acid(±)-2-(4-Isobutylphenyl)propanoic acid
Formula	:	$C_{13}H_{18}O_2$
Molecular weight	:	206.28 g/mol
CAS-No.	:	15687-27-1
EC-No.	:	239-784-6

Hazardous ingredients according to Regulation (EC) No 1272/2008

Component		Classification	Concentration
Ibuprofen			
CAS-No. EC-No.	15687-27-1 239-784-6	Acute Tox. 4; H302	<= 100 %

For the full text of the H-Statements mentioned in this Section, see Section 16.

SECTION 4: First aid measures

4.1 Description of first aid measures

General advice
Consult a physician. Show this safety data sheet to the doctor in attendance.

If inhaled
If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

In case of skin contact
Wash off with soap and plenty of water. Consult a physician.

In case of eye contact
Flush eyes with water as a precaution.

If swallowed
Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

4.2 Most important symptoms and effects, both acute and delayed
The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

4.3 Indication of any immediate medical attention and special treatment needed
No data available

SAFETY DATA SHEET

according to Regulation (EC) No. 1907/2006
Version 5.1 Revision Date 05.11.2018
Print Date 10.11.2018

SECTION 1: Identification of the substance/mixture and of the company/undertaking

1.1 Product identifiers

Product name	:	Ibuprofen
Product Number	:	I4883
Brand	:	Sigma
REACH No.	:	A registration number is not available for this substance as the substance or its uses are exempted from registration, the annual tonnage does not require a registration or the registration is envisaged for a later registration deadline.
CAS-No.	:	15687-27-1

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses	:	Laboratory chemicals, Manufacture of substances

1.3 Details of the supplier of the safety data sheet

Company	:	Sigma-Aldrich Company Ltd. The Old Brickyard NEW ROAD, GILLINGHAM Dorset SP8 4XT UNITED KINGDOM
Telephone	:	+44 (0)1747 833000
Fax	:	+44 (0)1747 833313
E-mail address	:	eurtechserv@sial.com

1.4 Emergency telephone number

Emergency Phone # +44 (0)870 8200418 (CHEMTREC)

SECTION 2: Hazards identification

2.1 Classification of the substance or mixture

Classification according to Regulation (EC) No 1272/2008
Acute toxicity, Oral (Category 4), H302

For the full text of the H-Statements mentioned in this Section, see Section 16.

2.2 Label elements

Labelling according Regulation (EC) No 1272/2008
Pictogram

Signal word	Warning
Hazard statement(s) H302	Harmful if swallowed.
Precautionary statement(s) P264	Wash skin thoroughly after handling.

SIGMA-ALDRICH

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SAFETY DATA SHEET

according to Regulation (EC) No. 1907/2006
Version 5.1 Revision Date 11.05.2017
Print Date 25.03.2019

SECTION 1: Identification of the substance/mixture and of the company/undertaking

1.1 Product identifiers

Product name : Oxybenzone

Product Number : 59647
Brand : Sigma-Aldrich
REACH No. : A registration number is not available for this substance as the substance or its uses are exempted from registration, the annual tonnage does not require a registration or the registration is envisaged for a later registration deadline.
CAS-No. : 131-57-7

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Manufacture of substances

1.3 Details of the supplier of the safety data sheet

Company : Sigma-Aldrich Company Ltd.
The Old Brickyard
NEW ROAD, GILLINGHAM
Dorset
SP8 4XT
UNITED KINGDOM

Telephone : +44 (0)1747 833000
Fax : +44 (0)1747 833313
E-mail address : eurtechserv@sial.com

1.4 Emergency telephone number

Emergency Phone # +44 (0)870 8200418 (CHEMTREC)

SECTION 2: Hazards identification

2.1 Classification of the substance or mixture

Classification according to Regulation (EC) No 1272/2008
Skin irritation (Category 2), H315
Eye irritation (Category 2), H319
Specific target organ toxicity - single exposure (Category 3), H335

For the full text of the H-Statements mentioned in this Section, see Section 16.

2.2 Label elements

Labelling according Regulation (EC) No 1272/2008
Pictogram

Signal word Warning

Hazard statement(s)
H315 Causes skin irritation.
H319 Causes serious eye irritation.

H335 May cause respiratory irritation.

Precautionary statement(s)
P261 Avoid breathing dust.
P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

Supplemental Hazard Statements none

2.3 Other hazards

This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

SECTION 3: Composition/information on ingredients

3.1 Substances

Formula : $C_{14}H_{12}O_3$
Molecular weight : 228.24 g/mol
CAS-No. : 131-57-7
EC-No. : 205-031-5

Hazardous ingredients according to Regulation (EC) No 1272/2008

Component		Classification	Concentration
Oxybenzone			
CAS-No.	131-57-7	Skin Irrit. 2; Eye Irrit. 2; STOT SE 3; H315, H319, H335	<= 100 %
EC-No.	205-031-5		

For the full text of the H-Statements mentioned in this Section, see Section 16.

SECTION 4: First aid measures

4.1 Description of first aid measures

General advice
Consult a physician. Show this safety data sheet to the doctor in attendance.

If inhaled
If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

In case of skin contact
Wash off with soap and plenty of water. Consult a physician.

In case of eye contact
Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.

If swallowed
Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

4.2 Most important symptoms and effects, both acute and delayed

The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

4.3 Indication of any immediate medical attention and special treatment needed

No data available

Hazard statement(s)

H315	Causes skin irritation.
H317	May cause an allergic skin reaction.
H319	Causes serious eye irritation.
H335	May cause respiratory irritation.

Precautionary statement(s)

P280	Wear eye protection/ face protection.
P280	Wear protective gloves.
P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P333 + P313	If skin irritation or rash occurs: Get medical advice/ attention.
P337 + P313	If eye irritation persists: Get medical advice/ attention.
Supplemental Hazard Statements	none

2.3 Other hazards

This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

SECTION 3: Composition/information on ingredients

3.1 Substances

Synonyms : 4-Amino-N-(5-methyl-3-isoxazolyl)benzenesulfonamide
N^1-(5-Methylisoxazol-3-yl)sulfanilamide

Formula : $C_{10}H_{11}N_3O_3S$
Molecular weight : 253.28 g/mol
CAS-No. : 723-46-6
EC-No. : 211-963-3

Hazardous ingredients according to Regulation (EC) No 1272/2008

Component		Classification	Concentration
Sulfamethoxazole			
CAS-No. EC-No.	723-46-6 211-963-3	Skin Irrit. 2; Eye Irrit. 2; Skin Sens. 1; STOT SE 3; H315, H317, H319, H335	<= 100 %

Hazardous ingredients according to Directive 1999/45/EC

Component		Classification	Concentration
Sulfamethoxazole			
CAS-No. EC-No.	723-46-6 211-963-3	Xi, R36/37/38 - R43	<= 100 %

For the full text of the H-Statements and R-Phrases mentioned in this Section, see Section 16

SECTION 4: First aid measures

4.1 Description of first aid measures

General advice
Consult a physician. Show this safety data sheet to the doctor in attendance.

If inhaled
If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

In case of skin contact
Wash off with soap and plenty of water. Consult a physician.

In case of eye contact
Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.

SAFETY DATA SHEET

according to Regulation (EC) No. 1907/2006
Version 5.1 Revision Date 22.01.2015
Print Date 25.03.2019

SECTION 1: Identification of the substance/mixture and of the company/undertaking

1.1 Product identifiers

Product name : Sulfamethoxazole

Product Number : S7507
Brand : Sigma-Aldrich
REACH No. : A registration number is not available for this substance as the substance or its uses are exempted from registration, the annual tonnage does not require a registration or the registration is envisaged for a later registration deadline.
CAS-No. : 723-46-6

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Manufacture of substances

1.3 Details of the supplier of the safety data sheet

Company : Sigma-Aldrich Company Ltd.
The Old Brickyard
NEW ROAD, GILLINGHAM
Dorset
SP8 4XT
UNITED KINGDOM

Telephone : +44 (0)1747 833000
Fax : +44 (0)1747 833313
E-mail address : eurtechserv@sial.com

1.4 Emergency telephone number

Emergency Phone # : +44 (0)870 8200418 (CHEMTREC)

SECTION 2: Hazards identification

2.1 Classification of the substance or mixture

Classification according to Regulation (EC) No 1272/2008
Skin irritation (Category 2), H315
Eye irritation (Category 2), H319
Skin sensitisation (Category 1), H317
Specific target organ toxicity - single exposure (Category 3), Respiratory system, H335

For the full text of the H-Statements mentioned in this Section, see Section 16.

Classification according to EU Directives 67/548/EEC or 1999/45/EC
Xi Irritant R36/37/38, R43

For the full text of the R-phrases mentioned in this Section, see Section 16.

2.2 Label elements

Labelling according Regulation (EC) No 1272/2008
Pictogram

Signal word Warning

SAFETY DATA SHEET

according to Regulation (EC) No. 1907/2006
Version 5.2 Revision Date 31.05.2017
Print Date 25.03.2019

SECTION 1: Identification of the substance/mixture and of the company/undertaking

1.1 Product identifiers

Product name	:	Triclosan
Product Number	:	PHR1338
Brand	:	Sigma-Aldrich
Index-No.	:	604-070-00-9
REACH No.	:	A registration number is not available for this substance as the substance or its uses are exempted from registration, the annual tonnage does not require a registration or the registration is envisaged for a later registration deadline.
CAS-No.	:	3380-34-5

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses	:	Laboratory chemicals, Manufacture of substances

1.3 Details of the supplier of the safety data sheet

Company	:	Sigma-Aldrich Company Ltd. The Old Brickyard NEW ROAD, GILLINGHAM Dorset SP8 4XT UNITED KINGDOM
Telephone	:	+44 (0)1747 833000
Fax	:	+44 (0)1747 833313
E-mail address	:	eurtechserv@sial.com

1.4 Emergency telephone number

Emergency Phone # +44 (0)870 8200418 (CHEMTREC)

SECTION 2: Hazards identification

2.1 Classification of the substance or mixture

Classification according to Regulation (EC) No 1272/2008
Skin irritation (Category 2), H315
Eye irritation (Category 2), H319
Acute aquatic toxicity (Category 1), H400
Chronic aquatic toxicity (Category 1), H410

For the full text of the H-Statements mentioned in this Section, see Section 16.

2.2 Label elements

Labelling according Regulation (EC) No 1272/2008
Pictogram

Signal word Warning

Hazard statement(s)

H315	Causes skin irritation.
H319	Causes serious eye irritation.
H410	Very toxic to aquatic life with long lasting effects.

Precautionary statement(s)

P273	Avoid release to the environment.
P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P501	Dispose of contents/ container to an approved waste disposal plant.
Supplemental Hazard Statements	none

2.3 Other hazards
This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

SECTION 3: Composition/information on ingredients

3.1 Substances

Synonyms	:	Irgasan5-Chloro-2-(2,4-dichlorophenoxy)phenol
Formula	:	$C_{12}H_7Cl_3O_2$
Molecular weight	:	289.5 g/mol
CAS-No.	:	3380-34-5
EC-No.	:	222-182-2
Index-No.	:	604-070-00-9

Hazardous ingredients according to Regulation (EC) No 1272/2008

Component		Classification	Concentration
5-Chloro-2-(2,4-dichlorophenoxy)phenol			
CAS-No. EC-No. Index-No.	3380-34-5 222-182-2 604-070-00-9	Skin Irrit. 2; Eye Irrit. 2; Aquatic Acute 1; Aquatic Chronic 1; H315, H319, H400, H410 M-Factor - Aquatic Acute: 100 - Aquatic Chronic: 100	<= 100 %

For the full text of the H-Statements mentioned in this Section, see Section 16.

SECTION 4: First aid measures

4.1 Description of first aid measures

General advice
Consult a physician. Show this safety data sheet to the doctor in attendance.

If inhaled
If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

In case of skin contact
Wash off with soap and plenty of water. Consult a physician.

In case of eye contact
Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.

If swallowed
Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

4.2 Most important symptoms and effects, both acute and delayed
The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

No components need to be disclosed according to the applicable regulations.

SECTION 4: First aid measures

4.1 Description of first aid measures

If inhaled
If breathed in, move person into fresh air. If not breathing, give artificial respiration.

In case of skin contact
Wash off with soap and plenty of water.

In case of eye contact
Flush eyes with water as a precaution.

If swallowed
Never give anything by mouth to an unconscious person. Rinse mouth with water.

4.2 Most important symptoms and effects, both acute and delayed
The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

4.3 Indication of any immediate medical attention and special treatment needed
No data available

SECTION 5: Firefighting measures

5.1 Extinguishing media

Suitable extinguishing media
Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

5.2 Special hazards arising from the substance or mixture
No data available

5.3 Advice for firefighters
Wear self-contained breathing apparatus for firefighting if necessary.

5.4 Further information
No data available

SECTION 6: Accidental release measures

6.1 Personal precautions, protective equipment and emergency procedures
Avoid dust formation. Avoid breathing vapours, mist or gas.
For personal protection see section 8.

6.2 Environmental precautions
No special environmental precautions required.

6.3 Methods and materials for containment and cleaning up
Sweep up and shovel. Keep in suitable, closed containers for disposal.

6.4 Reference to other sections
For disposal see section 13.

SECTION 7: Handling and storage

7.1 Precautions for safe handling
Provide appropriate exhaust ventilation at places where dust is formed.
For precautions see section 2.2.

7.2 Conditions for safe storage, including any incompatibilities
Store in cool place. Keep container tightly closed in a dry and well-ventilated place.

SAFETY DATA SHEET

according to Regulation (EC) No. 1907/2006
Version 5.5 Revision Date 03.07.2018
Print Date 10.11.2018

SECTION 1: Identification of the substance/mixture and of the company/undertaking

1.1 Product identifiers

Product name	:	Magnesium sulfate
Product Number	:	M7506
Brand	:	Sigma-Aldrich
REACH No.	:	01-2119486789-11-XXXX
CAS-No.	:	7487-88-9

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses	:	Laboratory chemicals, Manufacture of substances

1.3 Details of the supplier of the safety data sheet

Company	:	Sigma-Aldrich Company Ltd. The Old Brickyard NEW ROAD, GILLINGHAM Dorset SP8 4XT UNITED KINGDOM
Telephone	:	+44 (0)1747 833000
Fax	:	+44 (0)1747 833313
E-mail address	:	eurtechserv@sial.com

1.4 Emergency telephone number

Emergency Phone #		+44 (0)870 8200418 (CHEMTREC)

SECTION 2: Hazards identification

2.1 Classification of the substance or mixture

Not a hazardous substance or mixture according to Regulation (EC) No. 1272/2008.

2.2 Label elements

Not a hazardous substance or mixture.

2.3 Other hazards
This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

SECTION 3: Composition/information on ingredients

3.1 Substances

Synonyms	:	Magnesium sulphate
Formula	:	$MgSO_4$
Molecular weight	:	120.37 g/mol
CAS-No.	:	7487-88-9
EC-No.	:	231-298-2
Registration number	:	01-2119486789-11-XXXX