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Effect of salt impacted soils on the bioremediation of petroleum hydrocarbon compounds

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*“The past can hurt.
But the way I see it,
you can either run from it
or learn from it.”*

Rafiki, The Lion King

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Abstract

Continued growth of the population and global industrialization have placed an huge stress on ecosystems, releasing hazardous contaminants into the soil, endangering the environment and human health. Many industries and large population centers are concentrated in coastal areas. In fact, shorelines have a great influence from the natural intrusion of the sea, increasing the soil salinity.

The current work sought to address the problem of the soil remediation, previous studied by many authors, and take an approach to the salt effect on the bioremediation of soil contaminated with petroleum hydrocarbons compounds. At the end of this research, it was expected to make a comparative analysis of the bioremediation in saline and non-salted residual granitic soil.

For this purpose, a soil sample was salinized, simulating a concentration approximately equal to the salt concentration in sea water. Furthermore, the soil sample was used to perform two assays of assisted bioremediation with ventilation and without ventilation, contaminated with a mixture of benzene, toluene, ethylbenzene and ortho-xylene (BTEoX). Also, distribution study of the contaminants were made, with a saline sample and non-salted sample.

Contaminant analysis was performed by gas chromatography, in order to determine the concentration in the gas phase, allowing a study of the evolution of contaminant concentrations as a function of time. Concerning the assisted bioremediation with ventilation, respirometry was used in order to perceive the response of the microorganisms in the degradation of contaminants.

The obtained results, regarding the bioventing assay, were inconclusive. However, bioremediation assays revealed that soil salinity induced a negative effect on the degradation of contaminants, leading to the death of the consortium.

The study of contaminants distribution indicated that salinity did not have influence on the adsorption capacity. Finally, Freundlich model was the most appropriate isotherm model for the obtained results.

Keywords: soil, contamination, bioremediation, saline soils, adsorption.

Resumo

O crescimento contínuo da população e a industrialização global colocaram uma enorme pressão sobre os ecossistemas, libertando contaminantes nefastos no solo e que colocam, em risco, o meio ambiente e a saúde humana. Muitas indústrias e grandes centros populacionais estão, frequentemente, concentrados em áreas costeiras. De facto, as linhas costeiras têm uma grande influência da intrusão natural do mar, aumentando a salinidade do solo.

O presente trabalho procurou abordar o problema da remediação do solo, já muito estudado por diversos autores, e abordar o efeito do sal na biorremediação de solos contaminados hidrocarbonetos petrolíferos. O objetivo final da investigação, foi fazer uma análise comparativa da biorremediação em solos residuais graníticos salinos e não salinos.

Para este propósito, uma amostra de solo foi salinizada, de forma a similar uma concentração aproximadamente igual à concentração de sal na água do mar. Assim, a amostra de solo foi utilizada para realizar dois ensaios de biorremediação assistida com ventilação e sem ventilação, contaminados com uma mistura de benzeno, tolueno, etilbenzeno e orto-xileno (BTEoX). Além disso, foram realizados estudos de distribuição dos contaminantes, com as amostras salina e não salinas.

As análises foram realizadas por cromatografia gasosa, a fim de determinar a concentração na fase gasosa, permitindo um estudo da evolução das concentrações de contaminantes em função do tempo. Relativamente, à biorremediação assistida com ventilação, foi utilizada a respirometria para perceber a resposta dos microrganismos na degradação dos compostos orgânicos.

No que diz respeito à bioventilação, os resultados obtidos foram inconclusivos. Contudo, os ensaios de biorremediação revelaram que a salinidade do solo provocou um efeito negativo na degradação de contaminantes, levando à morte do consórcio.

O estudo da distribuição de contaminantes indicou que a salinidade não teve influência na capacidade de adsorção. Finalmente, o modelo de Freundlich foi o modelo de isotérmicas mais apropriado para os resultados obtidos.

Palavras-chave: solo, contaminação, biorremediação, solos salinos, adsorção.

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Abbreviations, Acronyms and Symbols

A1	- first assay
A2	- second assay
ATSDR	- Agency for Toxic Substances and Disease Registry
b	- maximum capacity of adsorption
BTEX	- benzene, toluene, ethylbenzene, xylene
BTEoX	- benzene, toluene, ethylbenzene, ortho-xylene
B2	- non-salted bioremediation assay 2
Bs1	- saline bioremediation assay 1
Bsb1	- saline control bioremediation assay 1
Bs2	- saline bioremediation assay 2
Bb2	- non-salted control bioremediation assay 2
Bvsb1	- saline control bioventing assay 1
Bvb2	- non-salted control bioventing assay 2
Bvs1	- saline bioventing assay 1
Bvs2	- saline bioventing assay 2
C_{aq}	- concentration of the contaminant in the aqueous phase
C_{eq}	- concentration of the contaminant in the fluid phase
C_{gas}	- concentration of the contaminant in the gas phase
C_p	- concentration of the pattern.
C_s	- concentration of the contaminant in the solid phase
CHC	- chlorinated hydrocarbon
CNS	- Central nervous system
DPE	- Dual-phase extraction
EC	- enrichment culture
ECD	- electron capture detector
ELCD	- electrolytic Conductivity Detector
EPA	- environmental Protection Agency
FID	- flame ionization detector
FPD	- flame Photometric Detector
GC	- gas chromatography
H	- Henry's Constant
I	- inoculum
IC	- inorganic carbon
K_{oc}	- organic carbon partition coefficient
K_{ow}	- octanol-water partition coefficient
K_s	- soil-water/ sediments-water partition coefficient
K_p	- partition coefficient
K_f	- Freundlich model constant
K_L	- Langmuir model constant

LB - Lysogeny Broth medium
 M_{aq} - mass of contaminant in the aqueous phase
 M_{gas} - mass of contaminant in the gas phase
 M_s - mass of contaminant in the solid phase
 M_{soil} - total mass of dry soil
 M_t - total mass of contaminant
MMA - liquid mineral medium A
MRL - minimal risk level
MS - mass spectrometer
n - constant of Freundlich
NAPL - non-aqueous phase liquid
NPD - nitrogen-phosphorus detector (NPD)
PAH - polycyclic aromatic hydrocarbons
PID - photoionization detector
q - amount of adsorbate per unity of absorbent
RFH - radio frequency heating
 S_b - solubility
S - bioventing column sampling port
S1 - bioremediation column sampling port
SC - soil sample from Carvalho, 2014.
SG - soil sample from Galp Energy, 2018.
SR - residual granitic soil
SRs - saline residual granitic soil
SVE - soil vapor extraction
SVOC - semi volatile organic compounds
T1 - first transfer
T2 - second transfer
TC - total carbon
TCD - thermal conductivity detector
TOC - total organic carbon
 V_{aq} - volume of contaminant in the aqueous phase
 V_c - volume of the individual contaminant
 V_{dp} - volume of the dry particles
 V_f - volume of Erlenmeyer flask
 V_{gas} - volume of contaminant in the gas phase
 V_t - total volume
 V_w - volume of water
VOC - volatile organic compounds
 W_s - water saturation content
 ρ - density
 ρ_c - density of the individual contaminants

σ - conductivity

1

Introduction

1.1 Framing and objectives of the study

Soil contamination by petroleum hydrocarbons is a current problem in the environmental context, not only at the portuguese territory, but also globally. Large scale petroleum production and intensive industrial activities has left behind a considerable amount of sites contaminated with organic and inorganic compounds.

Those contaminations can cause hazard to ecosystems and human health, by infiltrating in aquifers and being adsorbed to soil particle. However, removing organic pollutants in soil is possible due to the availability of biological technologies that deliver effective and low-cost results. Moreover, the petroleum components are, in the vast majority, biodegradable in aerobic conditions. In fact, there are a range of microorganisms, such as bacteria and fungi, capable of oxidate petroleum products efficiently, as is the case of BTEoX, a mixture of benzene, toluene, ethylbenzene and ortho-xylene, present in the gasoline (20%). In addition, the activity of microorganisms consortium depends on many external environmental factors and on the soil composition itself, which affects the capability to eliminate pollutants.

Portuguese territory is known for having an extensive coastline, where many central industries are concentrated, including two oil refineries. These industries are able to release hazards products that might effect the sustainability of the soil and contaminate the aquifers, compromising the public health. Furthermore, coastal zone is subject to a natural saline intrusion from the sea, which influences the physical and chemical properties of the soil, therefore, the capacity for bioremediation.

It is, from this perspective, that this research was developed. The main objective is to perform a comparative study of the bioremediation on saline soils contaminated by petroleum hydrocarbons compounds. Throughout this analysis, the study also will do a complementary research on the effect of salinity on sorption, since this mechanism is a retardation phenomenon of the contaminants and can influence directly its removal efficiency.

1.2 Organization of the dissertation

This dissertation is organized by five main chapters, bibliographic references and appendix. In chapter 1, the Introduction can be found, which includes the aims, the framing and the organization of the work.

Chapter 2 concerns the Context and State of the art. In this chapter a contextualization about the subjected is made, framing it globally and it is made a description of several topics concerning this study, such as the soil and its contaminations, soil rehabilitation technologies, the selected mechanisms used in the research, contaminants analyzed, among others.

Chapter 3 mentions the materials, reagents and samples used during the experimental work, as well as the methodology adopted for the research.

The results obtained in the remediation and sorption assays and its discussion can be found in Chapter 4.

Chapter 5 presents the final conclusions of the study.

Lastly, Chapter 6 will make a final assessment, indicating the objectives achieved, limitations and future work.

2

Context and State of the art

2.1 Soil

Soil performs many vital functions in the vast majority of ecosystems, since almost every forms of life depend on the soil for their survival. Soil is the support for plants which gives the necessary oxygen for the existence of life on Earth, it modifies the atmosphere through emissions and absorptions of some gases, such as carbon dioxide, methane and water vapor, it provides habitat for land animals and other living organisms. Moreover, it has an important role in the biogeochemical cycles, restoring the nutrients in ecosystems and it works as a reservoir and filter of water, as it absorbs, holds and purifies the water in terrestrial systems and filters the water before reaching the aquifers (Soil Science Society of America, 2010).

It is undoubtedly that the soil plays an important role on ecosystems maintenance and sustainability. However, as the population is growing along with industries, soil has been a target of extreme degradation, leading to huge consequences, not only at the environmental level, since it was verified impermeability situations, contamination, erosion and loss of organic content (Carvalho, 2014) but also at the economic level, in the sense that there are changes in production and development of the primary sector.

From an environmental perspective, soil can be defined as a non-renewable resource at the human scale of time, formed as a product of physical, chemical and biological erosion that acts on the terrestrial crust. It lies at the interface between lithosphere, biosphere, hydrosphere and atmosphere (Fiuza, 2009).

In addition, soil is a complex system with a multiphase nature. A solid phase formed by organic matter in different states of decomposition, from decaying remains of once-living things, due to microbial activity (Soil Science Society of America, 2010). This, not only increases the fertility, permeability to air and water, but also improves the soil structure. It is also formed by mineral particles, resulting from physical, biological and chemical erosion processes that disaggregate rocks into sediments of many sizes and different compositions, depending on the original rock. The liquid phase is made essentially of water, that runs freely through the particles and gas phase is formed by rarefied air, enriched by carbon dioxide (Carvalho, 2014). Commonly, the soil has a microbial phase, formed by living microorganisms, playing an important role on the maintenance of nutrients on the soil (Soil Science Society of America, 2010).

The mineral particles that incorporates the soil inorganic fraction are divided according to their equivalent diameter (Table 2.1).

Table 2.1: Soil classification according to the equivalent diameter of mineral particles

Category	Diameter (mm)	Description
Clay	< 0,002	Microscopic mineral particles of colloidal nature in layers
Silt	[0,002; 0,075]	Fine particles of minerals from the original rock
Sand	[0,075; 2]	Intermediate particles of minerals from the original rock
Gravel	[2 ;75]	Coarse particles of minerals from the original rock

The amount of clay, silt and sand imposes the textural group that soil belong. In order to determinate the soil textural classification, it is used the Feret diagram, as shown in figure 2.1.

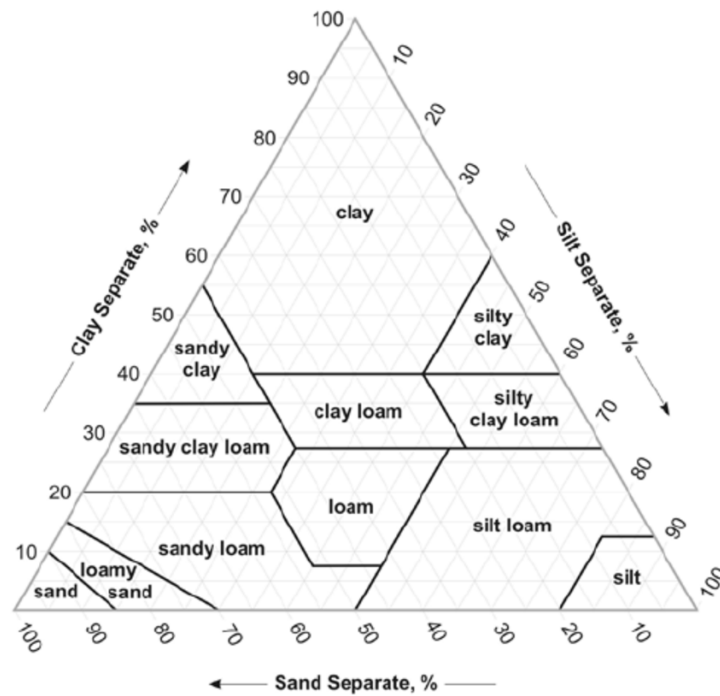


Figure 2.1: Feret Diagram. (Angima et al., 2018)

During soil formation, along the ages, soil created overlapped layers, usually called as horizons, each setting their own characteristics, making a soil profile. There are three main horizons that form the soil: Horizon A, B and C.

Horizon A, the top soil, is frequently grayish or black, due to the presence of humus and, also, vegetable and animals tissues in decomposition. As this layer is more superficial, it tends to be most affected by erosion, downwards movement to below layers and leaching of the soluble minerals by rain water (Fiuza, 2009). This is the soil layer where plants and organisms primarily

live.

Horizon B is commonly known as the subsoil. It is brownish and reddish, due to the high concentration of silicate clay, iron oxides, aluminum and carbonates, that comes from layers above and due to the erosion of A and B horizons itself. In addition, this layer is known as the illuviation zone, (Fiuza, 2009). Horizon C is formed by partially disaggregated material of underlying formations, where oxidation creates a color change (Fiuza, 2009). It is the deposit at Earth's surface from which the upper soil layers developed (Natural Resources Education, 2017).

In addition to those mentioned above, there are still two horizons that characterize the soil:

Horizon O, mostly formed by organic matter, such as decomposing leaves, above of A horizon. Some soil may not have this layer;

Horizon R, known as bedrock, is located under the C horizon and it is formed by a massive rock (Natural Resources Education, 2017).

Figure 2.2 shows an overview of the horizontal soil profile.

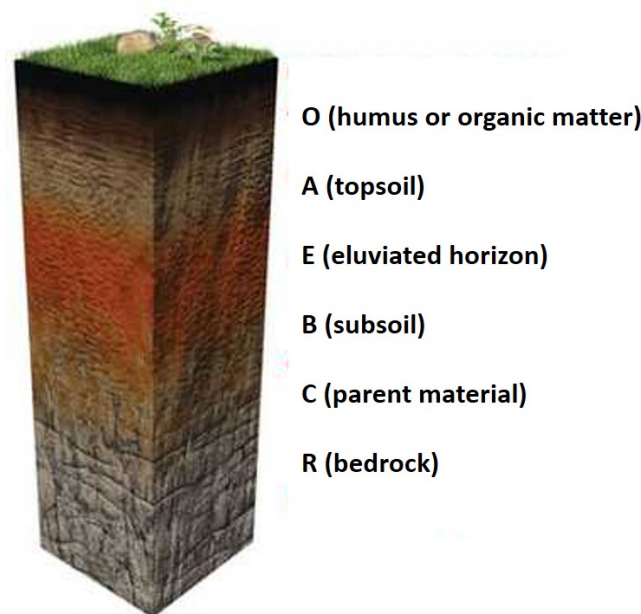


Figure 2.2: Horizontal Soil Profile. (Natural Resources Education, 2017)

2.2 Geological framing of Portugal

Concerning the geological context of Iberian Peninsula, it is integrated in the Meso-Europe Hercynica, one of the four main tectonic units that forms the European continent, essentially constitute by a Iberic Massif. This rocky mass, aged 200 millions years ago, forms a raised plateau, underlying the sea, in which sedimentary basins were subsequently installed. This massif is mainly composed by paleozoic and precambrian formations, with dominance of granitic batholiths, schists and quartzites. Besides, Hesperic Massif is divided by the Central Mountain

Rage into two blocks: North Meseta, drained by Douro river, and South Meseta, drained by Tejo, Sado and Guadiana rivers (Ferreira, 2000).

According to Ferreira (2000), there are two substantial geostructural units: the formations of the Iberian Massif, that occupies three-quarters of portuguese territory and post-Paleozoic shorelines, which are more recent lands from mesozoic and post-mesozoic.

Additionally, in the analysis of Iberian Massif (fig.3), it can be distinguished different geostructural units, such as Cantabrian zone, Austurian-Leonose zone and Central Iberian Zone, which includes a sequence of autochthonous and allochthonous: Schistose doma (CIZ) in Galicia-Trás-os-Montes, Ossa Morena zone and South Portuguese zone (Ferreira, 2000).

Figure 2.3 presents a view of the great units that compound the Iberian Peninsula, as well the tectono-stratigraphic scheme of Portugal.

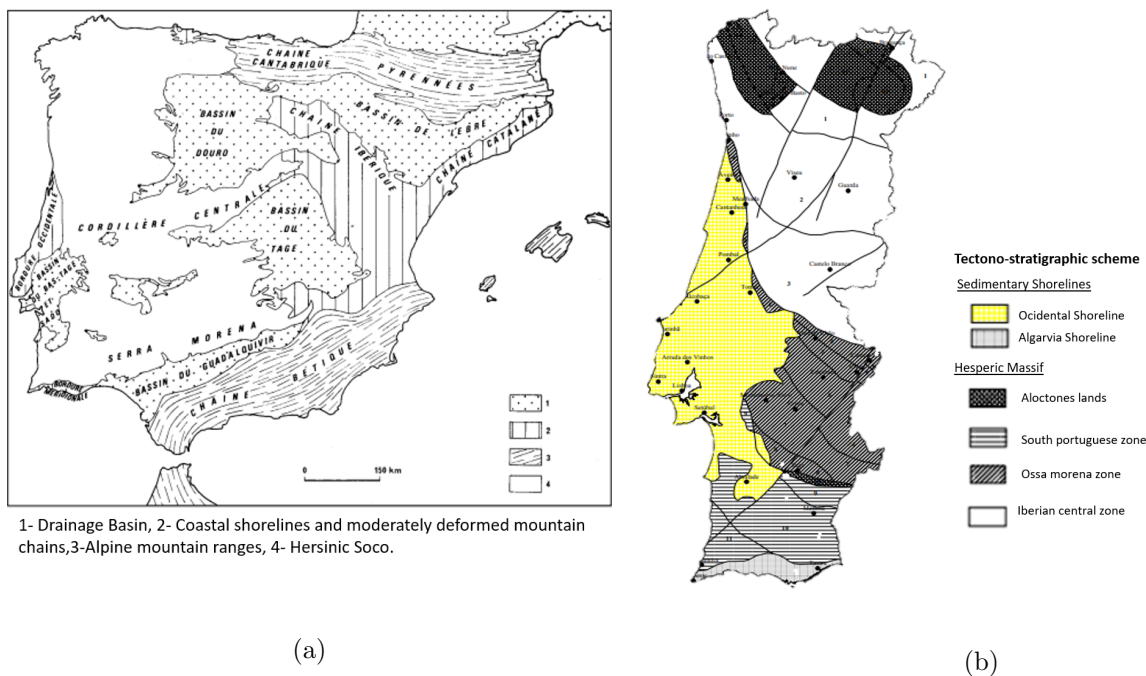


Figure 2.3: (a) Morphostructural units of Iberian Peninsula; (b) Tectono-stratigraphic scheme. (Ferreira, 2000)

Central Iberian Zone, in which Porto is included, is characterized for having regional metamorphism and abundant granite plutonism (Azevedo, 2013). The granitic formations in Porto, Braga and Guimarães are very heterogeneous and are essentially residual soils, products of granitic rocks weathering (Martins, 2003). The residual soil (SR) used for this research, resulting from the weathering, is a two-mica alkaline granite, medium grain, sometimes thin. This soil can be found in the geological context represented in figure 2.4, in Leça do Balio, Porto. It is a very altered geological material, in depth, with signs of intense feldspars kaolinization and oxidation (Carvalho, 2014).

The soil revealed a residual amount of feldspars, that are only shown in the coarser fraction, a reduced quartz content and an high amount of clay minerals. Mineralogical content, performed

by x-ray diffraction, revealed kaolinite (69-75%), moscovite (12-17%), montmorillonite (11%), quartz (6-16%), potassic feldspars (1%) and hematite (5%) (Carvalho, 2014).

The specific geological context of this residual soil justifies the unusual composition of this granite. Studies confirmed that the original rock was submitted to polycyclic transformations. Firstly, process of albitization and moscovitization. Then, periods of intensive kaolinization and, finally, quartz loss (Carvalho, 2014).

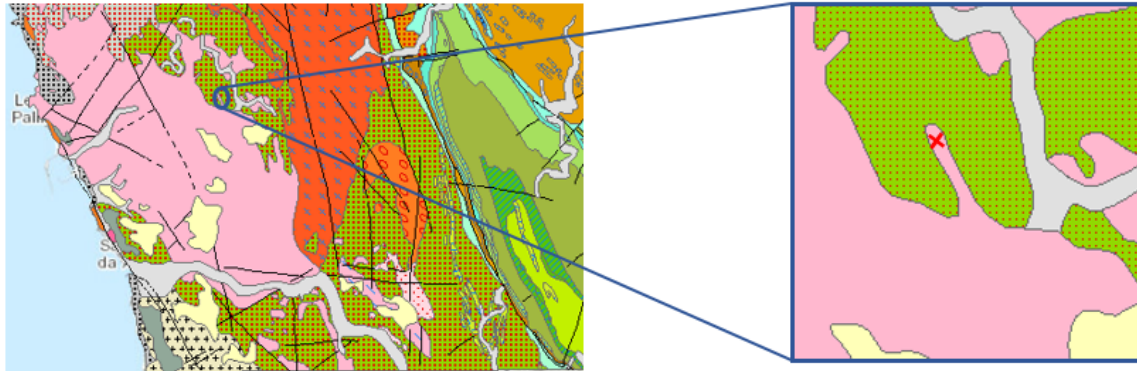


Figure 2.4: Location of the sampling of residual granitic soil (SR) in an excerpt of the Geological Map of Portugal at scale 1/200000 - Sheet 1 (Sistema Nacional de Informação Geográfica, 2018)

2.3 Impact of saline soils in Portugal

Steady increase of the soil salinity is one of the major causes of soil degradation worldwide. In Portugal, the problem is mostly extended to coastal areas from west to south (Gonçalves et al., 2015).

Salinisation leads to a great disturbance in biological, hydrological and biochemical earth cycles. High content of salinity can induce lost of emerging resources and services of the soil, affecting environmental conditions and agricultural production (Daliakopoulos et al., 2016).

Soils can be saline or sodic, endangering differently the soil systems. The process of sodicization is verified, when Na^+ ion is predominant in the soil exchange complex. Sodic soils present more harmful consequences for the environment compared to saline soils. In fact, the accumulation of Na^+ and Mg^{2+} results in the degradation of the soil structure and promote the clay dispersion, changing the pores geometry, which affects the intrinsic permeability and the water retention of the soil. In case of saline soils, the osmotic potential can be affected, reducing the water extraction capacity by plants (Gonçalves et al., 2015). Risk of salinisation are increased in soils with low permeability, affecting the leaching of soluble salts and temperate zone associated to high evapotranspiration rates and low precipitation.

Moreover, the causes of the salinisation can be either primary salinity or secondary salinity. In first place, primary salinisation correspond to the accumulation of salts due to natural processes, mainly including groundwater from maritime origin, direct movement of the sea tides

in lineshores, transport of maritime salt and physical and chemical weathering associated to transport from the parent rock. On contrary, secondary salinisation is introduced anthropogenically. Inappropriate use of the soil, ill-suited irrigation in soil having lower values of hydraulic conductivity and presenting lack of drainage systems, overuse of fertilizers in limited conditions of leaching are the main unnatural causes of salinisation (Daliakopoulos et al., 2016).

Globally, it is estimated that 10% of terrestrial surface is effect by salinisation phenomena, trending to be aggravated by human intervention and climatic change. As matter of fact, soil salinisation affects about 932.2 Mha worldwide, Europe presents salt impacted soil in the order of 30.7 ha. Particularly, portuguese territory, it is estimated around 150000 ha are affected by salinisation, in which 100000 ha are a result of primary salinity and 50000 ha as consequence of anthropogenic intervention.



Figure 2.5: Distribution of the salt impacted soil in Portugal (Daliakopoulos et al., 2016)

The contamination of saline soils is a current concern seldom studied. The high salt content can be an important factor in the remediation of contaminated soil with petroleum hydrocarbons. Theoretically, high salinity induces stress of high osmotic potential, resulting in the inhibition of the pollutants biodegradation and, some cases, the death of the living organisms (Qin et al., 2012).

2.4 Contamination of the soil

Global industrialization have placed an huge effort on ecosystems, specially on soil where activities are made in order to satisfy the human needs. These activities are releasing an amount of contaminants throughout the biosphere, endangering the soil with hazardous compounds (Carvalho et al., 2015). Nowadays, despite the high accessibility of renewable energies, they are not able to sustain the demand of global population growth, so fossil fuels are still widely used as a source to produce energy.

The large scale petroleum production and processing increases the likelihood of accidental spillage, which is one of the main causes of soil contamination (Shim et al., 2009), along with industrial production and municipal waste treatment (Carvalho, 2014). According to Organisation for Economic Co-operation and Development (OECD), in 2016, United States of American crude production was 447983 thousand of oil equivalent(toe), which means around 3.2 millions of barrel of oil equivalent(Mboe), while European Union decreased continuously their value of crude production to 68304 toe or 0.48 Mboe. Figure 2.6 demonstrates the evolution of crude oil production since the beginning of century.

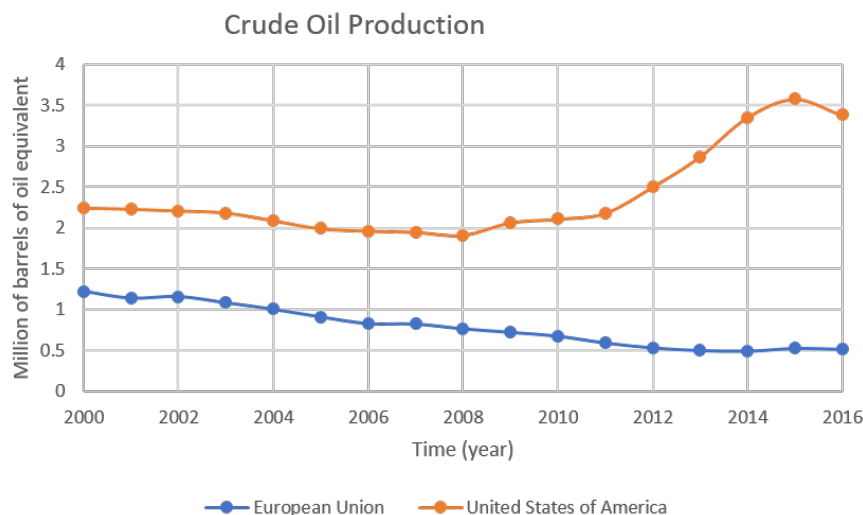


Figure 2.6: Oil crude production in European Union and United States of America. (OECD, 2016)

Soil contamination does not received the attention that should be given, despite its importance. In 2007, European Environment Agency stated that about 250000 sites were contaminated and needed remediation. Based on analysis made, it is expected that this trend continues in the future, and the total number of contaminated sites needing remediation might increase over 50% by 2025. Soil rehabilitation is advancing slowly, since nearly 80000 sites were clean-up, in the last thirty years.

Later, in 2013, European Comission, in a study published by Panagos, covering 33 countries,

stated that in nearly 1200 potentially contaminated sites, only 58 were remediated. Figure 2.7 confirms these values and, also, show a extrapolation of the potentially contaminated sites to 38 European countries.

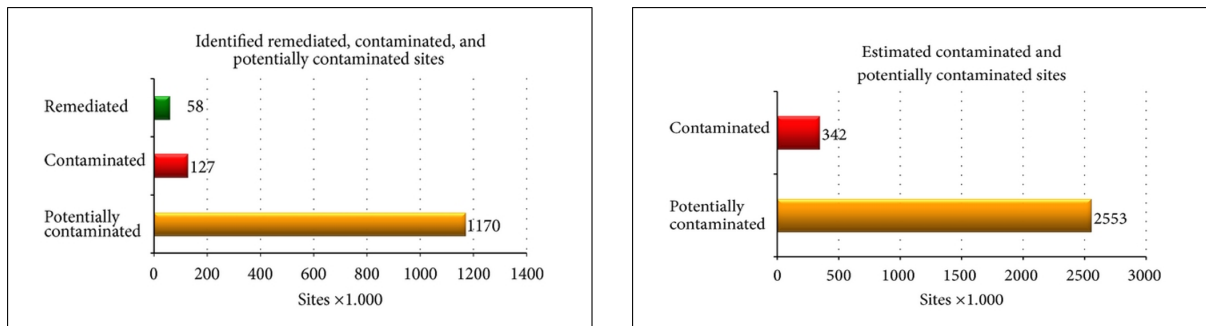


Figure 2.7: Contaminated Sites Statistics. (Panagos et al., 2013)

Soil contamination is mostly located close to waste landfills, industrial and commercial activities diffusing heavy metals, oil industry, military camps, and nuclear power plants (Panagos et al., 2013). Contaminated sites poses a significant environment hazard for terrestrial and aquatic system. Once the contaminants infiltrate ecosystems, they can start to pervade the food chain as well, which creates danger to human health. Figure 2.8 resumes the main contaminants affecting the soil.

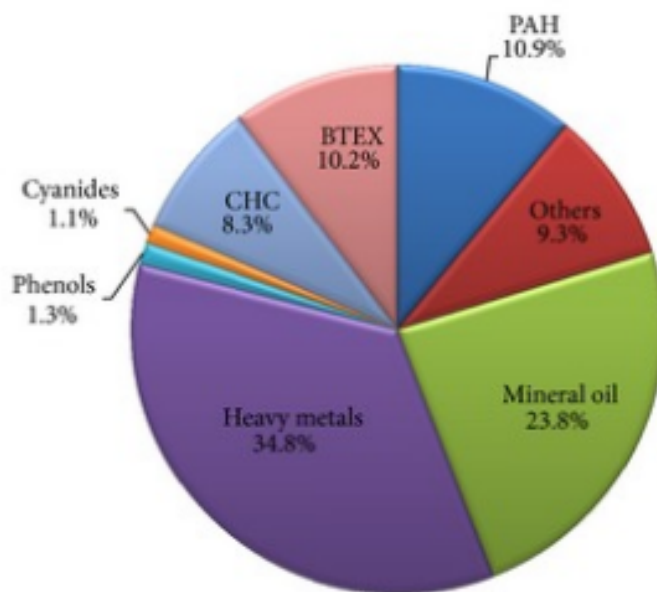


Figure 2.8: Overview of contaminants affecting soils (Panagos et al., 2013)

As is evidenced, the primarily contaminants on a contaminated site are heavy metals, mineral oil and organic products, such as BTEX and polycyclic aromatic hydrocarbons. It also shows that the currently situation is not ideal, which might be aggravated by the non-existence of official legislation by European Union.

Among organic products, it is particularly relevant to mention the benzene, toluene, ethylbenzene and three isomers of xylenes (ortho-, meta-, para-), known as BTEX or, in this dissertation case, BTEoX, since the xylene used was ortho-xylene. When a spill occurs in soil, these compounds are capable to be percolated throughout the vadose zone quickly, reaching the water table. They are monoaromatic hydrocarbons, which are the most soluble and mobile of the gasoline fraction, therefore, their ability to persist on soil particles for long time and the potential to bio-accumulate in living systems put these compounds as a priority for urgent treatment and remediation of the soil (Carvalho et al., 2015).

2.5 Rehabilitation of contaminated sites

Remediation of contaminated sites has been considered a sustainable assignment, regarding one of the aims for the sustainable development. Conserving the soil as a resource, can reduce the harm and risk on the human health and also prevent water, air and soil pollution. On the other hand, projects of remediation can have negative effects, such as high cost of execution and CO₂ production in some technologies. However, in the last decade, these effects have been counteracted as environmental awareness has increased (Al-Bukhaiti et al., 2017).

Selecting the most appropriate rehabilitation technology requires the consideration of some criteria, regarding the multiphasic complexity of the soil and other factors, such as the type of the contamination, characteristics of the soil and the contaminant, time and cost of the remediation strategy (Anderson, 2017).

Soil remediation technologies can be classified, according to the process in which they are based as: Physical, chemical, biological and thermal. Moreover, the application can be either made at the local (*in-situ*) or off-site (*ex-situ*).

Table 2.2 resumes the main soil rehabilitation technologies.

2.5.1 Physical Technologies

Physical based technologies are focused in the physical properties of the contaminants in order to retain or eliminate the contamination. The principle is to induce a phase transfer of the contaminant to another (Fiuza, 2009). The most used physical techniques are soil vapor extraction, multiphase extraction, electrokinetic, soil washing, air-sparging and air-stripping.

Soil Vapor Extraction (SVE) is applicable to volatile organic compounds (VOC) and semi-volatile organic organic compounds (SVOC). In this technology, a source of vacuum is generated, in order to create a pressure gradient, causing the air flow in the direction of the extraction points (Fiuza, 2009).

Multiphase extraction, commonly called DPE (Dual phase extraction), is an *in situ* tech-

Table 2.2: Overview of the soil rehabilitation technologies.

Soil Rehabilitation Techonologies			
Physical	Chemical	Biological	Thermal
• Vapor Extraction • Multiphase Extraction • Electrocnetic • Soil washing • Air-Sparging • Air-Stripping	• Soil Flushing • Chemical oxidation • Chemical reduction • Chemical extraction • Supercritical fluid extraction	• Bioventing • Biodegradation • Bio-sparging • Landfarming • Bio-piles • Natural Attenuation • Phyto rehabilitation	• Thermal desorption • Thermal destruction • STAR • Electrical Resistance Heating • Radiofrequency soil heating

nology, that allows the simultaneous removal, by pumping, of free organic compounds and hydrocarbon vapors. It is very efficient to remove not solubilized free organic phases, either in the saturated zone or in the infiltration zone (Fiuza, 2009).

Electrokinetic is an *in situ* technology applicable to homogeneous and moist soils. It is based on the application of low density electrical currents between electrodes placed on the soil, mobilizing the contaminants into an ionic form. This technology is often used to remove heavy metals or low permeability polar organic compounds, sludges and marine sediments.

Soil washing is applicable in excavated soils, commonly applied to remove radioactive, inorganic and organic contaminants. It has a great use as pre-treatment to reduce the volume of contaminated sites. The main goal is to concentrate the contaminated fraction of the soil.

Air sparging is intended to remove petroleum volatile organic compounds that are adsorbed to the soils and dissolved on the groundwater. It is made an air injection in the saturated, creating air bubbles that drag the contaminants. Simultaneously, a ventilation is made in the infiltration zone, allowing the compounds to volatilize.

Air stripping consists in a counter current mechanism between a flow rate of the aqueous phase with the dissolved contaminant and an air flow through a packed column. Inside this column, a high surface area is provided, allowing the transfer of the VOC to the gas phase.

2.5.2 Chemical Technologies

The purpose of the chemical based technologies is take advantage of the chemical properties, in order to modify the chemical structure, creating less toxic contaminants or even make them more easy to remove. Soil flushing, chemical oxidation and reduction, chemical extraction and supercritical fluid extraction are the most used chemical technologies in soil remediation.

Soil Flushing is a technology that aims to accelerate the contaminants mobilization, in order to collected them, so that they can be treated and recovered. It is used water or an aqueous so-

lution and injected, from the surface, promoting the dissolution of the pollutants and extracting them from the infiltration zone (Fiuza, 2009).

In the chemical oxidation, the main oxidants are sodium permanganate, Fenton's reagent and sodium persulfate. This technology is very useful to remove halogenated organic compounds (Carvalho, 2014).

Chemical reduction *in-situ* applies in contaminated soils remediation of halogenated ethanes and inorganic anions. It uses nano-particles, chemical reducing reagents, which allows their injection inside any plume of contamination, due to their small diameter (Carvalho, 2014), (Fiuza, 2009).

Supercritical fluids extraction technology uses fluids in high temperature and pressure conditions to dissolve organic compounds present in the soil, sediments or water. They are released from the super critical fluid through cooling and lowering the pressure. In case of the super critical water oxidation, an extremely rapid oxidation of the organic compounds is made, due to the forced contact between the air and the contaminated water (Fiuza, 2009).

2.5.3 Biological Technologies

Biological based technologies uses microbiota to degrade or transform hazardous pollutants into other products that do not involve such large dangers or even transform them in carbon dioxide, water, inorganic salts and microbial biomass (Environment Protection Authority 2005). The main biological technologies for soil treatment are: Bioventing, biodegradation, bio-sparging, landfarming, bio-piles, natural attenuation and phytoremediation (Fiuza, 2009).

Bioventing is a *in-situ* technology that uses indigenous microbiota, in order to biodegrade volatile organic compounds in the unsaturated zone. This technique induces an air flow, stimulating the bacteriological activity with or without nutrients addition. It can be similar to Soil Vapor Extraction, however the aim is to maximize the biodegradation, instead of the volatilization. Similarly, bio-sparging technology uses an oxygen supply to stimulate the microbial metabolism, however the application is made in the saturated zone.

Biodegradation is based on the capacity of the microorganisms to degrade contaminants. It can be anaerobic or aerobic and either intrinsic or enhanced. Intrinsic biodegradation relies on the autochthonous microbiota to naturally degrade the contaminants of the soils, without changing the current environmental conditions. The enhanced biodegradation is normally associated to biostimulation and bioaugmentation. Biostimulation is based on the stimulation of the the microbial activity by modification of the environment conditions, such as addition of oxygen, organic substrates, electron donors, nutrients and other factors that might affect the microbial metabolism (United States Environmental Protection Agency, 2006). Bioaugmentation uses indigenous microorganisms cultures that proved to be capable of degrading the target contaminants (Carvalho, 2014).

In anaerobic biodegradation, microorganisms metabolize different sources of carbon, originating inorganic compounds. The most promising technology regarding anaerobic biodegradation is anaerobic reductive dechlorination. Aerobic biodegradation transforms the organic compounds

into simple forms, such as carbon dioxide, water and biomass (United States Environmental Protection Agency, 2006).

Natural attenuation is included in the intrinsic biodegradation and is a passive alternative in which aims to use natural physical, chemical and biological processes of degradation and dissipation to eliminate contaminants of petroleum origin. Natural processes commonly used can include anaerobic and aerobic degradation, volatilization, dispersion, sorption, oxidation and reduction, biological reactions and stabilization (Fiuza, 2009), (United States Environmental Protection Agency, 2006).

Landfarming consists in spreading contaminated in small height piles, promoting the biodegradation through aeration, addition of nutrients and control of the moisture. Similar to landfarming, bio-piles uses similar mechanisms, however it has an air supply made through pipes under the piles (Fiuza, 2009).

Phytoremediation is based on the use of plants capable of remove, stabilize, transfer or destroy contaminants, through their metabolism. It involves a set of physical, chemical and biological processes that are influenced by plants, regenerating the contaminants. Plants are able to mineralize and immobilize toxic compounds in the rhizosphere, accumulating on the stems and leaves (Fiuza, 2009), (United States Environmental Protection Agency, 2006).

2.5.4 Thermal Technologies

Thermal technologies have been gaining credibly among other soil remediation techniques. Among them, it can be highlighted the following: Thermal desorption, thermal destruction, Sustaining Treatment for Active Remediation (STAR), electrical resistance heating and radiofrequency soil heating.

Thermal desorption and destruction are very useful in contaminated soils with petroleum hydrocarbons. It is based on the physical separation by induced temperature on the soil, causing contaminants desorption and, then, their volatilization. Formed gases are conducted to a gas stream and destroyed in a secondary burner or catalytic oxidation chamber (Corp, 1998), (Fiuza, 2009).

STAR is an efficient remediation approach to treat NAPL from the soil. This technique is based on smoldering combustion, which involves a flameless form of combustion, where oxidation reactions happen in order to provide the heat to destroy contaminants (Hasan, 2013).

Electrical Resistance Heating is based on a electric current placed in the soil. The resistance causes the soil and the fluids to heat up, removing the contaminants by direct volatilization and by air sparging (Carvalho, 2014), (TerraTherm, 2015).

Radiofrequency soil heating (RFH) is a technology in which the heat is made by electromagnetic radiation. RFH performs the heating at the molecular level, making this method very efficient. The technique itself can not remove the contaminants, however it enhanced the removal, when combined with other technologies such as vapor pressure, air-sparging, bioremediation, between others (Prince et al., 1999).

2.6 Distribution of contaminants by soil phases

Transport of soil contaminants are performed mostly by the groundwater movement (Fiuza, 2009), that circulates between the pores. Consequently, contaminants start to be transferred to different phases of the soil: solid, liquid and gas. Selecting the proper soil remediation technology must take in account how pollutants are distributed over the phases, since it conditions the contaminants availability to be removed. In addition, it is important to consider that when contaminants reach the soil, they are affected by several phenomena, such as retardation, attenuation, transport and increased mobility. These factors determinate the pollutants concentrations in the different phases, allowing to decide the most appropriate soil rehabilitation technology (Carvalho, 2014).

As already mentioned, soil is a really complex multiphase system, thus contaminants can be found in different compartments of this system, depending on the pollutants properties: Henry's constant, vapor pressure, solubility and partition coefficient. Partition coefficients are empiric constants that describe how a compound distributes itself between two phases; there are three main coefficients, with regard of soil remediation: octanol-water, soil-water and vapor-liquid. Moreover, organic contaminants can be found in the soil in the form of vapor in pores, a film, due to adsorption and dissolved in the aqueous phase, as well in the non aqueous liquid phase (NAPL) (Carvalho, 2014). Figure 2.9 presents how pollutants can be distributed and how their properties can influence the process.

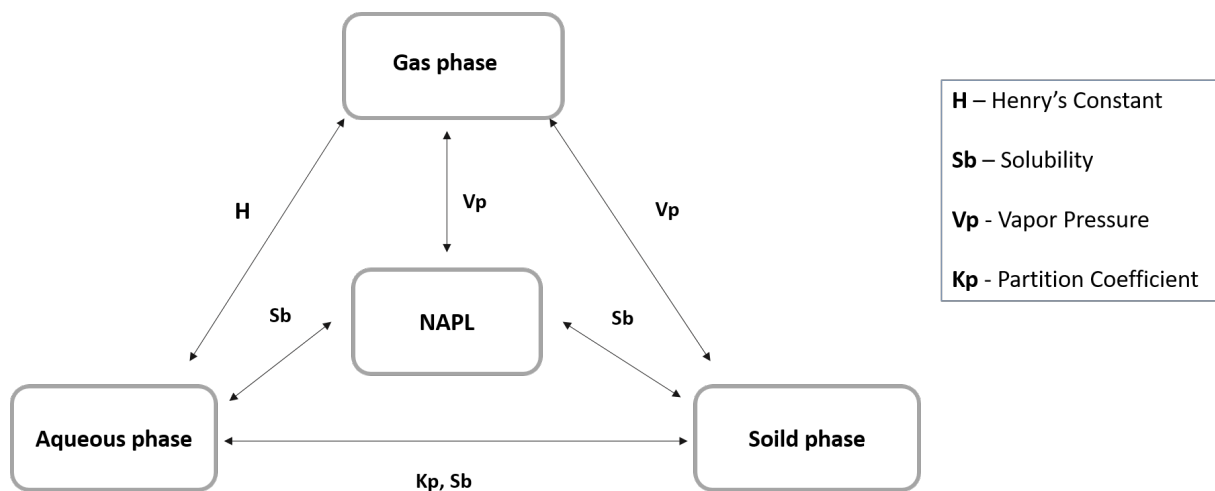


Figure 2.9: Distribution of the contaminants by different soil phases (Carvalho, 2014)

2.6.1 Liquid-gas partition

The physical and chemical properties of contaminants restricts how the distribution is made. In the context of the liquid-gas partition, Henry's constant, vapor pressure, solubility and octanol-water partition coefficient are parameters that are relevant to perform an accurate analysis (Carvalho, 2014).

Henry's constant (H) quantifies the partition between a gas phase and a non volatile liquid phase with a dissolved volatile constituent, therefore, it can be a indicator of volatility. In other words, it is a measure how the compounds in the soil moisture will volatilize. This constant depends on the nature of the gas and the solvent, as well the temperature. According to Henry's law, "At a constant temperature, the amount of a given gas dissolved in a given type and volume of liquid is directly proportional to the partial pressure of that gas in equilibrium with that liquid" (Sander, 2015), which means that at a constant temperature, in equilibrium state, the partial pressure of a volatile solute is directly proportional to its concentration at the solution, being normally estimated as the quotient between partial pressure and solubility (Fiuza, 2009). It is possible to investigate about the solubility of contaminants through Henry's constant value, in a sense that higher values of the constant correspond to a lower boiling point and a lower solubility. In opposition to this, a lower constant means an higher boiling point and solubility (Carvalho, 2014).

Solubility (S_b) can be defined as the capacity of a solute to dissolve in a pure solvent, which means that, at specified environmental conditions, it defines the maximum amount of solute that the solvent can hold within the solution (Gong et al., 2007). Substance with lower values of solubility tend to be more adsorbed, volatilized or tend to form a non aqueous liquid phase, instead of dispersing as a consequence of being dissolved in the soil moisture.

Octanol-water partition coefficient (K_{ow}) quantifies the sharing of an organic compound between an aqueous phase and an organic phase. Also, can indicate the amount of the pollutant that is retained by the living organisms. This parameters is commonly considered an indicator of hydrophobicity (Fiuza, 2009). A higher value of K_{ow} means that the substance is more hydrophobic, therefore, has more affinity to organic matter and can penetrate more easily in the tissues of the living organisms.

Finally, vapor pressure (V_p) of a substance is the pressure, in the equilibrium, between the gas phase and the substance in the solid or liquid state. It is a measure to determinate the capacity for a compound to evaporate, that is, to estimate the emission of volatile substances from the soil or water to atmosphere. Substances with higher values of vapor pressure ($>0,5$ mmHg) tend to be more volatilized than biodegraded (Fiuza, 2009).

2.6.2 Partition in the solid phase - Sorption

Sorption can be defined as chemical and physical interactions, in which a sorbate moves from a liquid phase and attach itself to solid phase (Fagundes et al., 2009). These attachments can occur with three main active parts: sorbate, which is the contaminant to be removed and moves between phases; sorbent, component that retain the sorbate; solvent, which is the substance

that dissolves the sorbate and is responsible for its transport (Fiuza, 2009). Soil sorption is affected by a given set of properties:

Characteristics of the solid matrix: organic matter content, texture, structure and mineralogy;

Characteristics of the fluid phase: pH, salinity and water content;

Properties of the contaminant to be removed: shape and size of the molecules, hydrophobicity, solubility, polarity and vapor pressure. (Carvalho, 2014)

Sorption is one of the main aspects that controls the contaminant concentration and its bioavailability in the soil. Investigate how this retardation mechanism affects the mobility and the amount of the pollutant available is important, because it can provide information about the extension of the contamination and the ecological impacts (Benetii et al., 2011). In addition, sorption is a crucial factor to understand the appropriate remediation technology to be selected (Carvalho, 2014).

Within the sorption, it can be noticed two different mechanisms: adsorption and absorption. In adsorption, sorbate is retained in the solid phase interface, for instance the surface of solid particles. On the other hand, in absorption, the solid phase assimilate and accumulate the sorbate inside the organic and inorganic particles (Fagundes et al., 2009).

Moreover, adsorption can be either physical or chemical, depending on the forces that bind the contaminant to the solid phase interface. Physical adsorption is generally a reversible process with low energy intermolecular forces, normally associated to Van der Waals forces. Also, in physisorption, the contaminant might condensate in the pores or even create multi layers in the adsorbent surface. On contrary, chemical adsorption is an irreversible process characterized for having high energy intermolecular forces, since there are permutations of electrons between the adsorbate and the adsorbent interface (Nascimento et al., 2014).

The intensity of a substance to distribute itself between the solid phase and the aqueous phase of the soil is determinant for its removal, as it is harder to remove a contaminant, when the sorption capacity is greater. The trend as a compound is adsorbed, can be estimated through the soil-water/sediments-water partition coefficient (K_s). This parameter can be determined by the ratio of the contaminant concentration in the soil/sediments and the contaminant concentration in water (Fiuza, 2009). Nevertheless, pollutants, as they are organic products, have apolar properties, so they are not often adsorbed by the mineral particles of the soil, but mainly by organic matter (Carvalho, 2014). In order to estimate the organic carbon partition coefficient (K_{oc}), i.e., the sorption of the organic compounds by the organic fraction of the soil, it is divided the contaminant concentration adsorbed by the organic carbon and the contaminant concentration in the water (Fiuza, 2009).

2.7 Contaminants distribution mathematical models

2.7.1 Adsorption isotherms

Mathematical models of adsorption use adsorption isotherms to conclude about the dependence of adsorbed substance amount of a determined concentration in the soil (Azevedo, 2013). Adsorption isotherms describe the variation of the concentration of the adsorbed substance with the concentration of that same substance in the fluid phase, in equilibrium and at constant temperature (Jorge, 2001).

According to Zhang, in 2016, there are six main types of adsorption isotherms that should be considered in the adsorption studies.

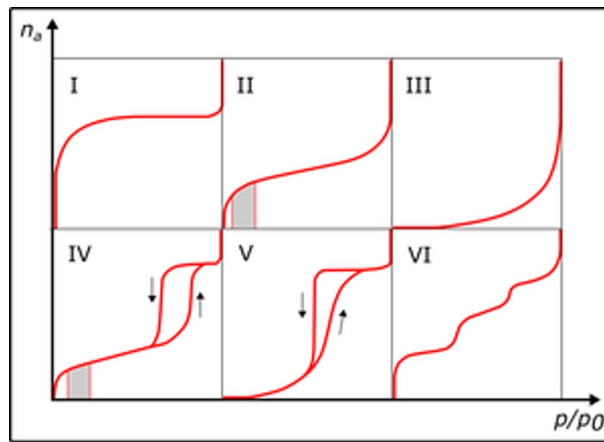


Figure 2.10: Types of adsorption isotherms (Connelly, 2017)

In the isotherm Type I, it can be verified a rapid increase of the amount of adsorbed gas in the solid phase with the increase of the relative pressure, in the equilibrium, until it reaches a saturation level (Amgarten 2006). The adsorption corresponds to a complete monolayer given by solid microporous which have relatively small external surfaces (Zhang, 2016).

Type II isotherm are characterized for non-porous or macroporous adsorbents and occurs very often in physical adsorption. It represents a system of multilayer formation, in which the first layer is more energetic than the successive layers. The inflexion point, marked by a darker zone in figure 2.10, indicates that the stage of the monolayer coverage is complete and, for higher concentrations, the layer condensation occurs (Carvalho, 2014), (Zhang, 2016).

Type III isotherm are formed by conditions in which the adsorbate molecules has stronger interactions with each other in comparison with the interaction between the adsorbent surface and the adsorbate, which means that the molecules has less affinity with the adsorbate interface (Zhang, 2016), (Amgarten, 2006).

Type IV isotherm have a similar beginning comparing to Type II isotherms, however it can be displayed a zone in which there is a small variation of the relative pressure, yet the adsorbed amount has a great increase. Also, it can be verified hysteresis loops, associated with capillary condensation, meaning the mesopores complete filling (Amgarten, 2006).

Type V isotherm are characterized by micro and mesoporous materials and weak adsor-

bent/adsorbate bonds. It can, also, occurs the complete filling of the pores (Amgarten, 2006), (Carvalho, 2014).

Type VI isotherm can be identified by the gradual adsorption of the multilayer, that, generally, occurs in uniform non-porous surfaces. Each level of the adsorption represents a complete monolayer (Carvalho, 2014), (Amgarten, 2006).

2.7.2 Adsorption isotherms methods of analysis

Nowadays, there are many methods of analysis to predict and evaluate the adsorption isotherms in the solid phase. The Langmuir and Freundlich models are the most widely cited and used in the soil sorption studies.

Freundlich model is an empiric model that describes the adsorption experimental results in heterogeneous multilayers of adsorption with an exponential distribution. Depending on the parameter, n , affinity between adsorbent and adsorbate, the isotherm can be linear, when it is verified that the n equals unity, which means there is only one layer. Otherwise, the energy distribution tends to vary with the adsorption density. Adsorption sites are more heterogeneous as long as the parameter n increases (Linhares et al., 2008), (Carvalho, 2014). Generally, the equation for the Freundlich models can be describes as follows in Equation 2.1.

$$q = K_f \cdot C_{eq}^n \quad (2.1)$$

where q is the amount of adsorbate per unity of absorbent, that is, the adsorbed amount in the soil, K_f is the adsorption coefficient of Freundlich, which represents the capacity of adsorbent to adsorb, C_{eq} is the concentration in the fluid phase, in equilibrium, and, lastly, as already mention, n is a constant representing the affinity between adsorbate and adsorbent.

Generally, it is possible to determine the coefficient K_f and n , through the logarithmic projection of the q versus C_{eq} . Thusly, the knowledge of the function allows to discover the parameter, which informs us about the surface heterogeneity and the adsorption intensity. Moreover, determining the constant K_f , which represents the ordinate, allows to conclude about the capacity of the adsorption (Linhares et al., 2008).

Langmuir model is a theoretical and non-linear model for adsorption with layers energetically homogeneous, where each molecule occupies one only site on the surface and considers the interactions between molecules insignificant. This model can provide a quantitative information that allows to discover the maximum amount adsorbed, as well as a qualitative data, regarding the bond energy (Linhares et al., 2008), (Carvalho, 2014). Langmuir model can be described by the equation 2.2 or 2.3:

$$q = \frac{K_L \cdot b \cdot C_{eq}}{1 + k \cdot C_{eq}} \quad (2.2)$$

or in the linearized form

$$\frac{1}{q} = \frac{1}{K_L \cdot b} \times \frac{1}{C_{eq}} + \frac{1}{b} \quad (2.3)$$

where q is the amount of adsorbate per unity of adsorbed, K_L is a constant regarding the bond energy between the adsorbed and the adsorbate, b is the maximum capacity of adsorption and C_{eq} is the concentration in the fluid phase, in equilibrium.

The graphic representation of the $\frac{1}{q}$ versus $\frac{1}{C_{eq}}$ allows to find the slope and the ordinate, which represents the parameters b and k .

Many researchers studied the adsorption of contaminants on soils and the factors that influence the process. According to Jorge, in 2001, sometimes it is difficult to conclude about the adsorption kinetics of organic compounds on soils, since many studies showed contrasting results. In some cases, the equilibrium can be achieved in a few hours of contact with the soil and that over 50% of the contaminants are adsorbed in a matter of a few minutes (Calvet, 1989). This is normally associated with a rapid and reversible adsorption. On the other hand, other studies have shown that the equilibrium can be achieved in months, depending on the water, type of soil and contaminants (Karickhoff, 1985).

Moreover, studies have shown that a significant amount of volatile organic compounds can be adsorbed directly from the gas phase in conditions of dry soils. Cases of low water content showed several orders of magnitude in the adsorption from the aqueous phase to the organic fraction of the soil. Also, as the water content increases, the order of magnitude decreases (Pennel et al., 1992).

Beyond this, an important aspect to be mentioned, considering the dissertation aim, is the salinity effect on adsorption. According to Hegemen (1995) and Hasan (1995), the adsorption in organic compounds is increased in soils with high content of salt, particularly in sediments. Cooney D. and Wijaya J., in 1989, also reported that sodium ions (salt ions), under particular pH conditions, increased the adsorption of dissolved organic matter and organic humic substances and the adsorption of synthetic dissolved organic materials was verified to be enhanced by calcium ions (Hasan, 2013). In contrast, Nelson and Yang (1995) stated that, at lower concentration (less than 5mM salt), the phosphate sodium effect on the adsorption of chlorophenol was not significant.

2.8 Contaminants

2.8.1 Benzene

Benzene is an aromatic hydrocarbon, liquid, clear, colorless and with sweet aroma. It is both volatile and flammable. Because it is an apolar molecule, it is slightly soluble in water (Morgan et al., 2009). Table 2.3 shows some of the chemical and physical properties of benzene.

Table 2.3: Benzene properties. Adapted from (Carvalho, 2014)

Benzene			
Chemical Formula	C ₆ H ₆	Vapor Pressure 25°C (mm Hg)	95.2
Molecular Mass (g mol ⁻¹)	78.11 g.mol ⁻¹	Density 20°C	0.8765
Apperance	Colorless liquid	Log Kow (a)	2.13
Odor	Aromatic. gasoline-like	Log Koc (b)	1.8 – 1.92
Melting Point	5.53°C	Henry constant 25°C (atm.m ³ mol ⁻¹)	5.48 × 10 ⁻³
Boilling Point	80.1°C	Solubility in water 25 °C (mg L ⁻¹)	1800

Benzene may arise naturally, but the major emissions are due to anthropogenic activities. It is primarily used in the manufacture of other organic chemicals, namely ethylbenzene, phenol, cyclohexane and aniline. Also very useful as a raw material to produce plastics, synthetic rubber and inks. It is a basic petrochemical, so is a crude oil constituent. Also, it is produced through catalytic reformat processes, gasoline pyrolysis, petroleum refining, biomass combustion, wastewater treatment, landfills, motor vehicle exhaust and steam crackers, between other industrial activities (ATSDR 2007).

Benzene is highly toxic and represent a huge risk to public health. According to Agency for Toxic Substances and Disease Registry (2007), benzene was considered carcinogenic to humans. It can cause leukemia, chromosomal modifications and affect blood cell production when chronic exposure. Besides, it cause headache, dizziness, drowsiness, confusion and loss of consciousness, eyes and skin irritant when acute exposure.

Table 2.4 resumes the Minimal Risk Level for benzene, along with exposure route, duration and the endpoint.

Table 2.4: Minimal Risk level for benzene (ATSDR, 2007)

Benzene	Route	Duration	MRL	Endpoint
	Inhalation	Acute	0.009 ppm	Immuno
		Intermediate	0.006 ppm	Immuno
		Chronique	0.003 ppm	Immuno
	Oral	Chronique	0.0005 mg/Kg/day	Immuno

MRL - Minimal Risk Level

The molecular structure of benzene is presented in the figure 2.11.

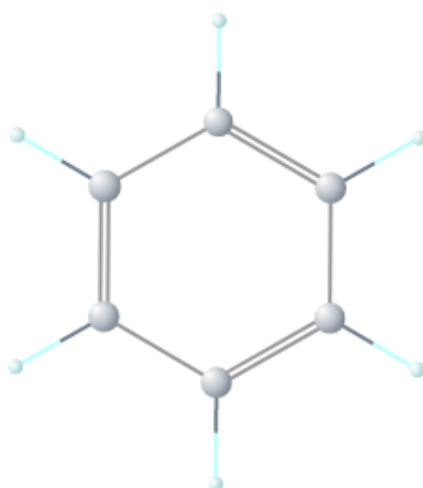


Figure 2.11: Benzene molecular structure. [Pubchem]

2.8.2 Toluene

Toluene is a volatile aromatic hydrocarbon, clear, colorless liquid with a distinctive smell. It is extremely flammable and it is not insoluble in water easily, but very soluble in organic solvents. Table 2.5 presents toluene physical and chemical properties.

Table 2.5: Toluene properties. Adapted from (Carvalho, 2014)

Toluene			
Chemical Formula	C ₇ H ₈	Vapor Pressure 25°C (mm Hg)	28.4
Molecular Mass (g. mol ⁻¹)	92.14 g·mol ⁻¹	Density 20°C	0.8669
Apperance	Colorless liquid	Log Kow (a)	2.72
Odor	Sweet pungent, benzene-like	Log Koc (b)	2.06-2.3
Melting Point	-95°C	Henry constant 25°C (atm·m ³ mol ⁻¹)	6.74 × 10 ⁻³
Boillling Point	111°C	Solubility in water 25 °C (mg·L ⁻¹)	490

Toluene appears in the environment mostly by anthropogenic activities, although it appears naturally in crude oil and tolu tree. It is very commonly used for synthesis of benzene, paint thinners, fingernail polish, nylon, plastics, as a chemical solvent and, also, as added to gasoline. Toluene is mainly released from gasoline processing, due to eventual spillage and traffic emissions, which constituted a huge risk for humans health (Agency for Toxic Substances and Disease Registry, 2000). According to U.S Department of Health and Human Services, in 2017, a monitoring data of outdoor air in the United States showed that the average concentrations of toluene is 1-35 parts per billion (ppb) by volume (ppbv).

Acute and chronic exposure to toluene is extremely alarming, considering that the central nervous system (CNS) is the primary target organ feeling any kind of damage. In case of acute exposure by inhalation, it is frequently observed central nervous system dysfunction and narcosis to low and moderate levels of toluene, and symptoms including fatigue, sleepiness,

headaches and nausea. At higher levels of exposure, CNS depression, cardiac arrhythmia and death have occurred. It was also reported, in case of ingestion, effects of constriction and necrosis of myocardial fibers, swollen liver, congestion and hemorrhage of the lungs, and tubular kidney necrosis.

Chronic effects can be drowsiness, ataxia, tremors, cerebral atrophy, involuntary eye movements, impaired speech, hearing and vision. Also, it was observed inflammation and degeneration of the nasal and respiratory epithelium. At high levels of toluene, it cause central nervous system depression (Agency for Toxic Substances and Disease Registry, 2000).

Table 2.6 shows the Minimal Risk Level for toluene.

Table 2.6: Minimal risk level for toluene (ATSDR, 2000).

Toluene	Route	Duration	MRL	Endpoint
	Inhalation	Acute	2 ppm	Neuro
		Chronique	0.003 ppm	Neuro
	Oral	Acute	0.8 mg/Kg/day	Neuro
		Chronique	0.2 mg/Kg/day	Imuno

The molecular structure of toluene is presented in the figure 2.12.

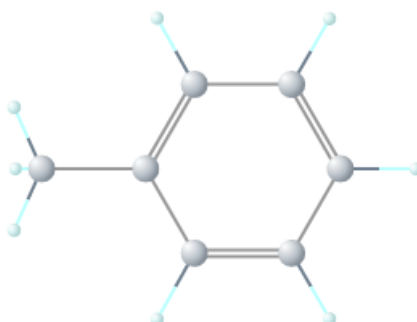


Figure 2.12: Toluene molecular structure. [Pubchem]

2.8.3 Ethylbenzene

Ethylbenzene is a colorless liquid with an aromatic odor, volatile, flammable and slightly soluble in water. It is an aromatic hydrocarbon that occurs naturally in petroleum and fuels (ATSDR, 2010).

Table 2.7 presents the general properties of ethylbenzene.

Table 2.7: Ethylbenzene properties. Adapted from (Carvalho, 2014)

Ethylbenzene			
Chemical Formula	C ₆ H ₅ – C ₂ H ₅	Vapor Pressure 25°C (mm Hg)	9.6
Molecular Mass (g. mol ⁻¹)	106.17	Density 20°C	0.867
Apperance	Colorless liquid	Log Kow (a)	3.15-4.34
Odor	Gasoline-like	Log Koc (b)	1.98-2.65
Melting Point	-95°C	Henry constant 25°C (atm.m ³ mol ⁻¹)	8.68 × 10 ⁻³
Boilling Point	136°C	Solubility in water 25 °C (mg.L ⁻¹)	152

Ethylbenzene is used as a solvent in the production of synthetic rubber and it is used primarily as a precursor in the production of styrene. It is also used as a constituent of asphalt and naphtha, as well as in manufacture of acetophenone, cellulose acetate, diethylbenzene, ethyl anthraquinone. The release of this chemical occurs due to oil, gas and coal burns from industries and other manufacture processes. Also, it can be found near to crude storage tanks (ATSDR, 2010).

Ethylbenzene can be found in soil by gasoline spills and not appropriate disposal of industrial and household wastes. It can contaminate the groundwater, since it can move very easily through soil pores (ATSDR, 2010).

The acute exposure can lead to throat and eyes irritation, chest constriction and some neurological effects, such as dizziness. When the exposure is chronic by inhalation, in low concentration, it can be observed some potentially irreversible damage to the inner ear, hearing of animal, as well as kidney damage. Also, the long-term exposure may cause cancer in humans (ATSDR, 2010). Table 2.8 shows the minimal risk level for ethylbenzene.

Table 2.8: Minimal Risk Level for ethylbenzene (ATSDR, 2010)

Ethylbenzene	Route	Duration	MRL	Endpoint
	Inhalation	Acute	5 ppm	Neuro
		Intermediate	2 ppm	Neuro
		Chronique	0.06 ppm	Neuro
	Oral	Intermediate	0.4 mg/Kg/day	Hepatic

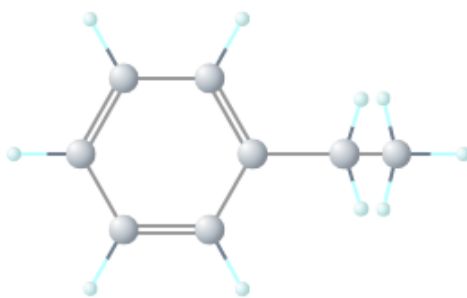


Figure 2.13: Ethylbenzene molecular structure. [Pubchem]

2.8.4 Xylene

Xylene can be distinguished by its isomers: meta-xylene, para-xylene and ortho-xylene. These isomers are characterized for having methyl groups in different positions on the benzene ring, causing them to assume other properties (fig. 2.14). Generally, xylenes are flammable liquids, colorless, insoluble and with a sweet odor (ATSDR, 1995).

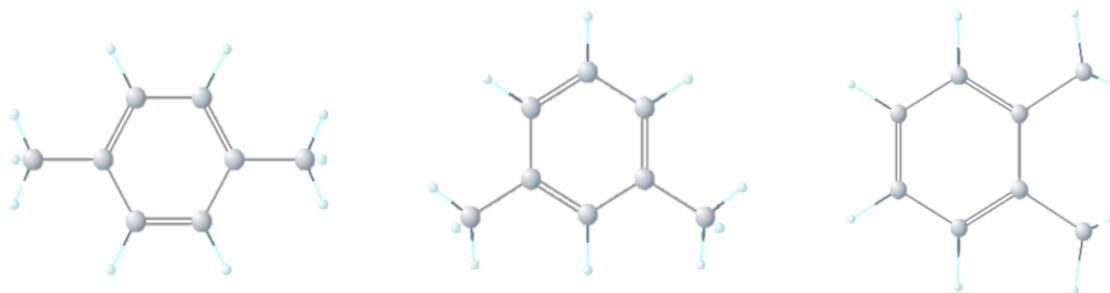


Figure 2.14: Xylenes isomers molecular structure. From left to right: p-xylene, m-xylene, o-xylene. [Pubchem]

Table 2.9 resumes the isomers and mixed xylene properties.

Table 2.9: Xylenes properties (adapted from (ATSDR, 1995))

	Mixed Xylene	meta-xylene	para-xylene	ortho-xylene
Chemical Formula	C ₈ H ₁₀	C ₈ H ₁₀	C ₈ H ₁₀	C ₈ H ₁₀
Molecular Mass (g mol ⁻¹)	106.16	106.17	106.17	106.16
Apperance	Clear	Colorless	Colorless	Colorless
Odor	Sweet	Sweet	Sweet	Sweet
Melting Point	No data	-47.6°C	13.3°C	-25°C
Boilling Point	158.5°C	139.2°C	138.37	144.4°C
Vapor Pressure 25°C (mm Hg)	6-16	6	5	6.5
Density 20°C (mg/L)	0.864	0.864	0.88	0.861
Log Kow (a)	3.12-3.20	3.2	3.12	3.15
Log Koc (b)	-	2.22	2.11	2.31
Henry constant 25°C (atm.m ³ mol ⁻¹)	-	0.00766	0.00519	0.00766
Solubility in water 25 °C (mg L ⁻¹)	130	146	178	198

Xylene is an aromatic hydrocarbon (Fig. 2.14) that arise naturally from the petroleum. It can be found in leakage emissions from industry sources, traffic, since it is present in small amounts in gasoline. It is used as an organic solvent, cleaning agent and varnishes. Also, can be used as a material in the plastic, chemical and synthetic fiber industries. Xylene isomers are frequently used to produce polymers, such as plastics (ATSDR, 1995).

Studies showed that mixed xylene or xylene isomers affected basically in similar ways, despite of some isomers may have different potentialities to produce notorious effects. In cases of acute inhalation exposure, it was observed dyspnea, nose and throat irritation; some gastrointestinal effects might be gastric discomfort, nausea, and vomiting. Besides, impaired short-term memory and reaction time, performance decrements in numerical ability and alterations in equilibrium and body balance are some cases of neurological effects in humans. When dermal exposure occurs, it was verified transient skin irritation and dryness, scaling of the skin.

In cases of chronic exposure, data showed some neurological effects, such as headache, dizziness, fatigue, tremors, incoordination, inability to concentrate, impaired short-term memory and, finally, anxiety. Beyond that, impaired pulmonary function, severe chest pain, increased heart palpitation and possible effects on the kidney (ATSDR, 1995).

Table 2.10 presents the Minimal Risk Level for xylene.

Table 2.10: Minimal Risk Level for Xylene (ATSDR, 1995)

Xylenes	Route	Duration	MRL	Endpoint
	Inhalation	Acute	2 ppm	Neuro
		Intermediate	0.6 ppm	Neuro
		Chronique	0.05 ppm	Neuro
	Oral	Acute	1 mg/Kg/day	Neuro
		Intermediate	0.4 mg/Kg/day	Neuro
		Chronique	0.2 mg/Kg/day	Neuro

2.9 Selected technologies

As mentioned in section 2.5.2, remediation of soils contaminated with petroleum hydrocarbons can be accomplished by different approaches. Biological technologies are environmentally sound and cost-effective method, when applied in soils containing organic compounds (Environment Protection Authority, 2005).

Remediation of soil by biotechnologies is based on the mineralization of the contaminants into water, carbon dioxide, inorganic compounds or reduced toxicity organic compounds. Moreover, biodegradation of hydrocarbons in contaminated sites is performed by microorganisms, such as bacteria and fungi. The organisms that present this catabolism are most often found close to polluted soils (Galdames et al., 2017). Physical and chemical factors are required in order to increase the bioremediation efficiency, as is the case of the minor and major nutrients, aerobic conditions, temperature, pH, water (Iturbe et al., 2015).

The present study will focus in two different approaches: assisted bioremediation involving augmentation and biostimulation with and without venting. Venting assays were designated by bioventing and non-ventilated trials were designated by bioremediation. The main difference is the supply of oxygen that is administrated in the bioventing assays.

Bioventing is an *in-situ* technology widely used in the removal of petroleum products, where indigenous microorganisms are used to biodegrade organic substances adsorbed to soils and sediments, usually in the unsaturated zone. In fact, this technology increases the efficiency by inducing air flow into the vadose zone, through extraction and injection wells and, also, by adding nutrients, in certain cases. The mechanism itself is very similar to SVE (Soil Vapor extraction), however this process promotes the volatilization of the contaminants, instead of biodegrading them. On the other hand, bioventing uses a smaller air flow to promote the biodegradation of the pollutants and minimize the volatilization (United States Environmental Protection Agency, 2006).

Figure 2.15 shows an accurate scheme of how bioventing is performed in a pilot scale.

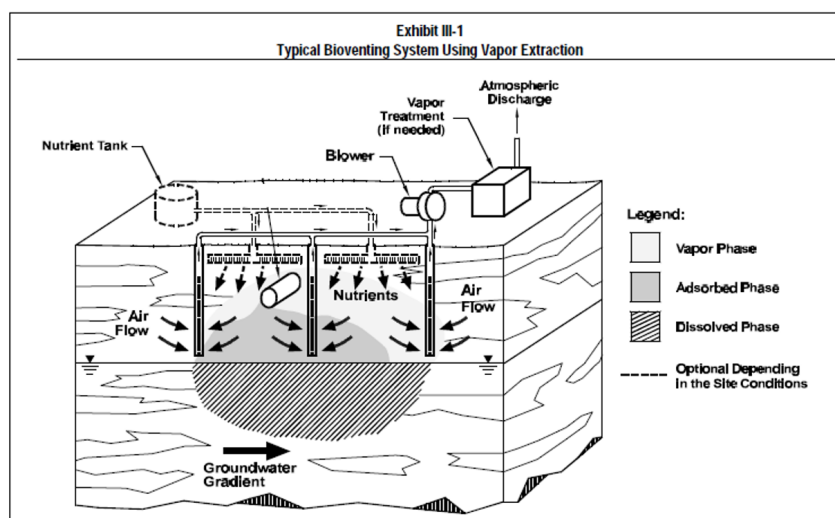


Figure 2.15: Scheme of bioventing systems *in-situ*. (Environmental Protection Agency, 2017)

2.10 Respirometry

Respirometry is a technology capable of measuring the biological activity, in terms of the oxygen consumption and carbon dioxide production. Among the applications fields, such as Biology, Biotechnology, Environment, Agronomy, etc., respirometry also can be applied to soil remediation with petroleum products, allowing to evaluate the biodegradation kinetic and evaluate the influence of other factors that might affect the process, such as nutrients addition (Vila, 2004). The ventilation setting in the remediation of soil, it is important to consider the ideal air flow used. If the flow is too high, it is more difficult to control and oxygen and carbon dioxide oscillations. On the other hand, a low flow may not ensure the necessary oxygen for the biological activity (Carvalho, 2014).

2.11 Gas Chromatography

After the World War II, petrochemical industry evolved very quickly, demanding more efficient and accurate samplings. Gas chromatography replaced almost every technologies capable of analyzing related hydrocarbons, since GC was able to reduce the amount of gas sample and the required time to performed the analysis (Ettre, 2004).

Gas chromatography (GC) is a technology used worldwide for analyzing almost every kind of organic compounds, gaining a particular importance to analyze petroleum hydrocarbons (Piantanida et al., 2014), (Ettre, 2004). It is a separation technique which evolved two phases: the stationary phase and the mobile gas phase. The stationary phase can be both solid or liquid. During the separation, the sample to be analyze is vaporized and dragged by the carrier gas, which represents the mobile phase, to the column. The principle of the separation in a chromatographic process is based on the different vapor pressure of the substances and affinity with the stationary phase (Piantanida et al., 2014). After the analysis is made, the system reports a set of peaks that represents an area that can be converted to concentrations.

Gas chromatography technique can provide a good resolution of the results, with sharp and symmetric peaks and an high repeatability and reproducibility of the retention times. Also, GC can present an high precision and accuracy in quantification based on measurements of the peak area (Al-Bukhaiti et al., 2017).

Additionally, there is a wide range of detectors that can be used in gas chromatography, according to the specifications of the substances to be analyzed. So, it can be distinguished: Flame Ionization Detector (FID), Nitrogen Phosphorus Detector (NPD), Electron Capture Detector (ECD), Thermal Conductivity Detector (TCD), Flame Photometric Detector (FPD), Photoionization Detector (PID), Electrolytic Conductivity Detector (ELCD) and Mass Spectrometer (MS) (Yu et al., 2014).

Flame Ionization Detector (FID) is one of the most used in this analyzing technique. It has a great use in the petrol and food industry, since it is highly sensitive to volatile organic compounds, organic substances containing hydrocarbons and, also, other substances, such as methane, ethane, etc (AGA, 2017).

Figure 2.16 shows a simple scheme of the chromatographic components.

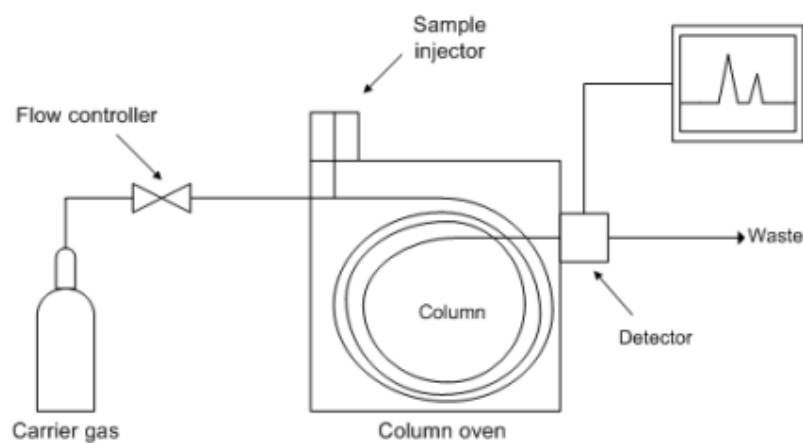


Figure 2.16: Schematic diagram of the GC components. (Piantanida et al., 2014)

3

Materials and Methods

3.1 Materials

3.1.1 Equipments

The equipment used during the experimental work is resumed in the Table 3.1.

Table 3.1: Equipment used during experience

Equipment	Brand	Model
Autoclave	All American Panasonic	25X MLS-3020U
Analytical Scale	Chyo	JL-180
Semi-Analytical Scale	Kern	EW1500-2M
Thermostatic chamber	WTW	TS1006-i
Conductivity meter	WTW	Inolab
Gas Chromatograph	Shimazu	GC-2010
Stove	Binder	FD
pH meter	WTW	Inolab
Respirometer	Servomex	MiniMP 5200
Orbital Stirrer	Heidolph	Unimax2010
Mass/Flowmeter GFC	AALBORG	GFC17
Syringes	Hamilton ILS (10, 50, 250, 2500 μ L)	----
Erlenmeyer Flasks	Schott (500, 1000, 2000 ml)	----
Mininert® Valves	----	----
Stainless Steel Columns	----	----
TOC-VSCN analyser	Shimadzu	SSM-500A



(a)



(b)



(c)



(d)

Figure 3.1: Equipments used. (a) Respirometer Servomex, (b) Flow Controller, (c) Gas Chromatograph, (d) Syringes

In figure 3.1, it is presented the respirometer (Servomex, MiniMP 5200), which records the amounts of oxygen and carbon dioxide in the air that flows through the device in the moment of the analysis. It works in conjunction with the flowmeter, that gives us precision on the flows between 0 to 20 mL/min (Carvalho, 2014). Also it is presented a gas chromatograph (Shimadzu, GC-2010) equipped with a TRB-5 capillary column (Teknokroma) with dimensions of 30 m x 0.25 mm x 0.25 μ m. There were two kinds of syringes, one for gases, specially used to pick up the contaminant in the headspace and inject in the GC and another for liquids that were used to inject the contaminant in the Erlenmeyer flask, as well as in the analysis columns.

For the assays, a total of five columns of stainless steel were used, two of them with dimensions of ϕ 10 cm (inner diameter) x 50 cm (inner height) used for bioventing tests, and the remaining three had the same diameter but an inner height of 35.5 cm. Those were used for bioremediation tests.

The highest reactor had 4 entries. Two for air outlet(O) and inlet(I) (top and bottom), and two at 2/3(sampling port (S)) and 1/3 of the lateral height (port 2 (E)). The smallest reactor had 4 entries, as well, however only one port at the top (port 1 (E1)) and the other three at the side wall (2/3(S1), 1/3(E2) and near to the bottom (E3)). Figure 3.2 presents a view of the

each columns.

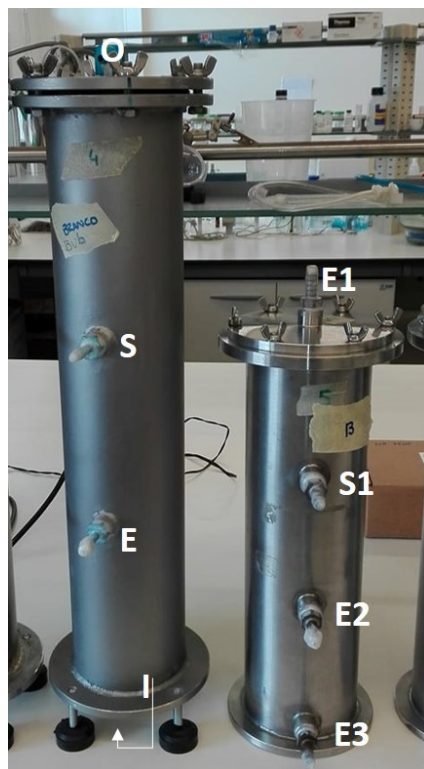


Figure 3.2: Stainless Steel Columns entries

3.1.2 Reagents

The reagents used during the experimental work are summarized in Table 3.2. They are divided by name, brand and concentration and, also, where they were used for.

Table 3.2: Reagents used during work

Used for	Reagents	Brand	Concentration
Contaminant	Xylene	Fisher Scientific	99.7%
	BTEoX	---	25% volume per contaminant
	Benzene	Panreac	99.5%
	Toluene	Panreac	99.5%
	Ethylbenzene	Merk-Schuchardt	> 99%
	Ortoxylyene	Petrogal	---
Liquid Mineral Medium A	CaCl ₂ .H ₂ O	Merck	0.47 mmol/L
	NaCl	Fisher Scientific	0.14 mmol/L
	KNO ₃	Merck	1.2 mmol/L
	NaNO ₃	Merck	8.2 mmol/L
	MgSO ₄ .7H ₂ O	Merck	0.41 mmol/L
	NTA	Merck	0.52 mmol/L
	FeSO ₄ .H ₂ O	Merck	2 mg/L
	ZnSO ₄ .7H ₂ O	Merck	0.1 mg/L
	MnSO ₄ .5H ₂ O	Panreac	0.03 mg/L
	H ₃ BO ₃	Merck	0.3 mg/l
	CoSO ₄ .7H ₂ O	Panreac	0.24 mg/L
	CuSO ₄ .5H ₂ O	Merck	0.01 mg/L
	NiSO ₄ .2H ₂ O	Merck	0.02 mg/L
	Ca(OH) ₂	Merck	0.5 mg/L
	EDTA	Panreac	5 mg/L
	NaMoO ₄ .2H ₂ O	Merck	0.03 mg/L
	(NH ₄) ₂ SO ₄	Merck	3.8 mmol/L
Lysogeny Broth (LB medium)	KH ₂ PO ₄	Merck	4.0 mmol/L
	NaHPO ₄	Merck	6.0 mmol/L
	Tryptone	Labkem	10 g/L
	Yeast Extract	Liofilchem	5 g/L
	Agar Powder	Himedia	20 g/L
Saline Solution	HCl	---	0.1 M
	NaCl	Fisher Scientific	10 g/L
	CaCl ₂	Merck	0.4 g/L
	KCl ₂	Applichem	0.4g/L
	NaCl	Fisher Scientific	10 g/L

3.2 Methods

During experimental work, two main assays were made (April-May and June-August) for assisted biodegradation with venting (Bioventing) and assisted biodegradation without venting (Bioremediation). For those, it was necessary to use a specific consortium, to perform the degradation of the contaminants. Table 3.3 resumes the assays by its designation, contaminant used and the start and end date.

Assays	Designation	Contaminant	Start date	End date
Assay 1	A1	BTEoX	April 2018	May 2018
Assay 2	A2	BTEoX	June 2018	August 2018
Inoculum	I1	Xylene	26-03-2018	07-04-2018
	I2	O-xylene	02-05-2018	14-05-2018
	I3	O-xylene	02-05-2018	14-05-2018
	I4	O-xylene	15-05-2018	30-05-2018
	I5	O-xylene	18-05-2018	26-05-2018
	I6	O-xylene	18-05-2018	26-05-2018
	I7	O-xylene	30-05-2018	09-06-2018
	I8	O-xylene	30-05-2018	09-06-2018
	I9	O-xylene	30-05-2018	09-06-2018
	I10	O-xylene	01-06-2018	10-06-2018
	I11	Benzene	01-06-2018	10-06-2018
	I12	Benzene	11-06-2018	19-06-2018
	I13	Benzene	11-06-2018	20-06-2018
	I14	Benzene	12-06-2018	21-06-2018
	I15	Benzene	12-06-2018	21-06-2018
	I16	Benzene	16-06-2018	31-06-2018
Bioremediation 1	Bs1	BTEoX	07-04-2018	02-05-2018
	Bb1	BTEoX	07-04-2018	02-05-2018
Bioremediation 2	Bs2	BTEoX	21-06-2018	02-08-2018
	B2	BTEoX	21-06-2018	11-07-2018
	Bb2	BTEoX	21-06-2018	02-08-2018
Bioventing 1	Bvsb1	BTEoX	07-04-2018	02-05-2018
	Bvs1	BTEoX	07-04-2018	02-05-2018
Bioventing 2	Bv2	BTEoX	21-06-2018	25-07-2018
	Bvs2	BTEoX	21-06-2018	25-07-2018

Table 3.3: Resume of the assays.

3.2.1 Sampling

The soil sample used in the remediation tests was a residual granitic soil (SR) collected, in 2010, from a digging between 2 to 3 meter deep. It was used a backhoe loader and a metal shovel for the extraction. The local coordinates are 41°12'37.4"N 8°37'46.8"W, Leça do Balio, Porto (3.3). The soil was air dried and stored at room temperature, protected from the light, according to Carvalho (2014).



Figure 3.3: Location of the origin of granitic soil sample (Leça do Balio-Porto 41°12'37.4"N 8°37'46.8"W)

3.2.2 Characterization of the soil

The soil was submitted to physical and chemical characterization. The parameters pH, carbon content, organic matter content and the particle size distribution were performed by the School of Engineering of Polytechnic of Porto (ISEP-IPP).

pH

For pH determination, different methods were made. In first place, according to the methodology used by Carvalho, in 2014, it was used a beaker with 20 g of soil and 20 ml of distilled and demineralized water. The solution was placed under constant stirring for 15 minutes and then 20 minutes resting. After this, the pH was measured by immersing the pH electrode in the clear supernatant. This method also allowed to determinate the conductivity, with the help of a conductivity meter. The method used by ISEP-IPP laboratories consisted of weighing 10g of dry and sifted soil (<2mm) into a recipient with 25 ml of ultrapure water. Also, the assays were made separately with KCl 1 mol/l and CaCl₂ 0.01 mol/l. In each cases, the solution was agitated for a few minutes and left to rest. The reading was made with the help of a calibrated pH meter.

Bulk density

The bulk density (ρ) is directly influenced by the soil weight and its apparent volume, as it corresponds to the ratio between weight and volume. It is affected by the degree of compaction

and mineralogical composition (Schon, 2011). In order to determine the bulk density, the soil was freely dropped into a 250 ml graduated cylinder, so that it can not experience any kind of compaction. Thus, it was divided the soil weight used and the volume occupied, determining the soil bulk density.

Chemical composition

The elemental chemical composition was made through X-Ray fluorescence method.

The determination of the total carbon (TC) and the inorganic carbon was performed with the help of a TOC carbon analyzer. The TOC parameter was determined by the difference between TC and IC.

Water Content

Determining the saturation water content required weighting 200 g of dry soil into a beaker, adding distilled and demineralized water moderately until it performs a saturated soil paste. It can be considered saturated, when the paste slides on the goggle and presents a shinny surface (Carvalho, 2014). Then, the solution rested for one hour to measure the new weight. Any excess of supernatant water must be remove and, then, be noted the new solution weight. A content is calculated dividing the difference between the dry weight and the final weight with the dry weight.

Particle size

Particle size distribution was made by sieving test, recommended by European Standard EN ISO 17892-4:2016 for particle larger than 0.063 mm, followed by the use of a laser granulometer for the fine fraction characterization.

Cationic exchange capacity

Cationic exchange capacity determination was made by an expedited method for two different samples. One was a salted residual soil (SRs) and the other one was a non-salted residual soil (SR). Firstly, 2 g of soil was weighted into a 50ml beaker. Then, it was added 5 ml of sodium pyrophosphate and 25 ml of distilled and demineralized water. It was agitated for five minutes, while it was prepared a burette filled with 10 ml of methylene blue. After that, the solution pH was adjusted to 3.5, with the help of hydrochloric acid 1 M. Then, the beaker was placed under the burette and was slowly dropped the methylene blue into the solution. After each administration, the solution was agitated for two minutes and, then, with a pasteur pipette was collect a small sample and dropped on a filter paper. When it was verified a blue-greenish aura, the volume spent on the experiment was noted and cationic exchange capacity was calculated through equation 3.1.

$$CEC = \frac{V_t \cdot C_b}{M_{ds}} \quad (3.1)$$

where, CEC is the cationic ion capacity, in mol/Kg, V_t is the volume of methylene blue used, in ml, and M_{ds} is the mass of dry soil used, in Kg.

Organic matter

The organic matter content was performed by loss on ignition (LOI) which is widely used to measure the soil organic matter content (Nelson, 1995). According to the Benton Jones methodology (2001) a ashing vessel and the dry sample of soil were weighted. The set was putted inside of a drying oven for four hours, at the temperature of 400°C. After this intervention, the ashing vessel with the ashes was weighted. The organic content was determinate by the equations 3.2 and 3.3.

$$ma = m_{va} - m_v \quad (3.2)$$

$$MO(\%) = \frac{m_{ds} - m_a}{m_{va}} \times 100 \quad (3.3)$$

where ma is the ashes mass (g), m_{va} is the ashing vessel plus ashes mass (g), m_v is the ashing vessel mass (g), MO is the organic matter content (%), m_{ds} is the mass of sample of dry soil(g).

3.2.3 Artificial salinisation of the soil sample

As mentioned in chapter 1, the aim of this work is to ascertain the impact of salt on bioremediation of the soil. Therefore, it was prepared 7.35 kg soil sample, for assay 1 (A1), that was submitted to three cycles of salinization. Each cycle involved moisten the sample with 2 liters of saline solution (10g/l NaCl, 0.4 g/l KCl, 0.4 g/l CaCl₂), without saturating the soil and precipitating the salt (fig.3.4). Between each salinization, the soil sample was left to dry for 48h in the oven at the temperature of 50°C. Initially, the sample was dry for 24 hours at the temperature of 105°C.

For the assay 2 (A2), it was prepared 6 kg of granitic soil, which was submitted to the same cycles of salinization, except for the initial dryness.

Besides, to completely study the salt effect, 3 kg of non-salted soil was prepared and dried. This allowed us to compare the bioremediation and bioventing at the two possible cases. Also, the results obtained on the PhD thesis by Carvalho, 2014, will help to conclude and compare the results.



Figure 3.4: Soil sample after irrigation with saline solution

When the salinisation cycles were finished, the soil was divided, with the help of a riffle sample splitters (fig.3.5), in four portions (A1) and five portions (A2), each portion for a different analysis column.

Table 3.4: Scheme of soil sample used in the column test

	Assay 1	Assay 2
Saline soil	2x1500g	1x1600g
	2x1136g	1x1136g
Non-salted soil	-	1x1600g
	-	1x1136
	-	1x743g



Figure 3.5: Riffle sample splitter

To ensure that during the experiment there was no autochthonous microorganisms or any kind of contamination, all prepared soil samples were sealed in different bags and submitted to

three sterilization cycles (Fig.3.6). Each cycle was performed on the autoclave for 15 min, 121°C and 1 atm. The soil rested 24h before the next sterilization cycle.



Figure 3.6: Sterilized bags of granitic soil

3.2.4 Preparation of inoculum

Bioremediation and bioventing are only effective if there are microorganisms capable of degrading the selected contaminants, as they use it as a carbon source for their growth. For this matter, it is important to extract and isolate the microorganisms, so that they can be properly adapted. The adaptation must be done gradually, so successive transfers were made from the enrichment culture, to fresh mineral medium, in order to grow the consortium.

For the microbial consortium, two different contaminated soils were tested. The first one (SG) was collected, with the help of a metal shovel, from the refinery of Matosinhos. The soil was fresh and had recently suffered a oil spillage from the pipes. The sample was collected in three different points, in order to increase the likelihood of having the necessary microbial population. The green point (Fig. 3.7) represents the point where the inoculum presented more relevant results, since it was closer from the spillage source. Second one (SC), was a soil that has been used in previous developed research works (Carvalho, 2014).

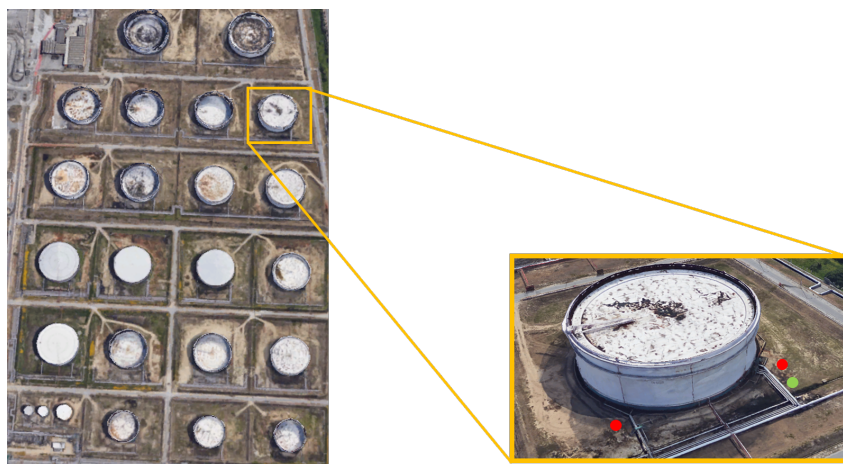


Figure 3.7: Scheme of soil extraction, around the storage tank in the sampling area

The enrichment cultures (EC) were prepared in Erlenmeyer flasks (1000 ml and 2000 ml), closed with Mininert® valves. As shown in figure 3.8, inoculum assays were performed in three different ways. Scheme 1 represents the inoculum 1 (I1), as designated in the table 3.3, that was used for first assay (A1). The enrichment culture took 20g of soil SC and 200 ml of liquid mineral medium A (MMA). The medium composition is described on the table 7, on section 3.1.2. EC from scheme 2 was made with 100 ml of MMA and 10 g of SG (from I2 to I11). Finally, the third method, scheme 3, took the same amounts, but with soil SC. Each enrichment culture was incubated, during 24 hours, in orbital shaker (130 rpm) inside of the thermostatic chamber.

It was necessary to make successive transfers, so that the consortium could be well adapted. In this sense, the culture was transferred to new Erlenmeyer flask, considering a ratio of EC:MMA 5:95 (%v/v). At this point, it was necessary to add a carbon source: the contaminants. The level of contamination for the first two schemes was 240 $\mu\text{l/l}$, so it was added 70.2

μl of xylene and 48 μl of ortho-xylene. Third test used a contamination level of 200 $\mu\text{l/l}$ of benzene.

The concentrations in the gas phase were monitored through gas chromatography, until it detected values below 0.5 mg/l in the headspace, which meant that T1 was ready to be transferred. In second transfers, a ratio of EC:MMA 50/50(%v/v) was used. Therefore, first test took 300 ml of MMA, 300 ml of T1 and 96 μl , and for the remaining tests was added 200 ml of MMA and T1. In relation to the carbon source, it was added 96 μl and 80 μl of o-xylene and benzene, respectively.

During all the process, cultures from I1 to I11 were on the orbital shaker at the velocity of 130 rpm, at the temperature of 26°C. The assays with benzene, from I12 to I16, were incubated at the temperature of 28°C.

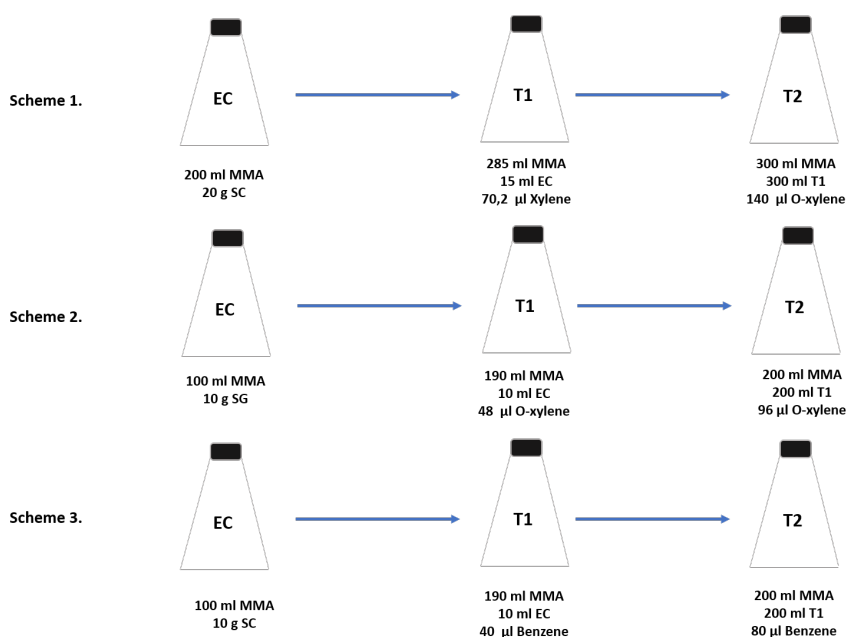


Figure 3.8: Scheme of the inoculum assays.

3.2.5 Quantification of the biomass

Before inoculating the columns with the consortium, the biomass present was quantified, through successive dilutions. For the first dilution, 1 ml of inoculum was transferred to a test tube containing 9 ml of saline. After that, from the same test tube, 1 ml was withdrawn and transferred to another test tube. The procedure was repeated four times, making a dilution of 10^{-4} . Between each dilution, the test tube was placed on the vortex for brief seconds, homogenizing the inoculum on the tube.

Then, with the aid of a micropipette, 100 μl of the inoculum of each dilution was spread separately on different Petri plates, containing mineral medium LB (table 3.2). The plates were incubated during 72 hours inside of the thermostatic chamber. At the end of the third day, the biomass of each dilution was counted. All the process was made in an aseptic space with the bunsen beak on.

Lastly, the final quantification of the colony-forming units per milliliter was made by the equation 3.4:

$$CFU/ml = B \times Fd \quad (3.4)$$

where CFU/ml is the quantification of colony-forming unit per milliliter in each dilution, B is the counted biomass in the Petri dishes, Fd is the dilution factor used and V_{tube} is the volume of the solution in the test tube.

Figure 3.9 illustrates the procedure of the dilutions and the biomass spread in plates.

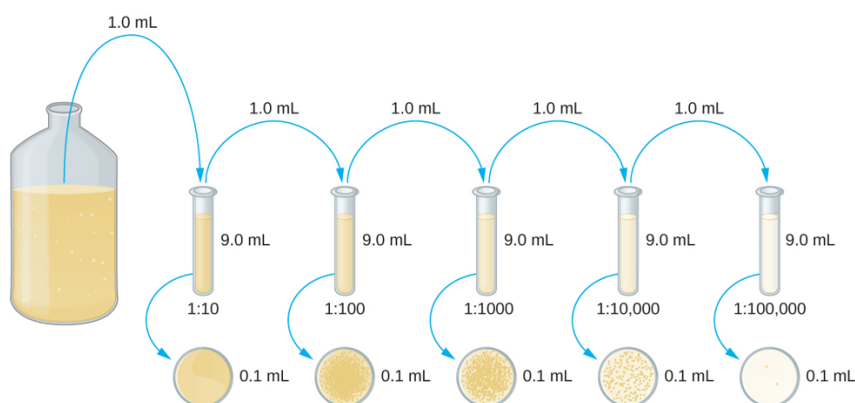


Figure 3.9: Scheme of the dilution of the inoculum (OpenStax, 2006)

3.2.6 Bioremediation and Bioventing Assays

Bioventing assays were all performed on stainless steel columns with 50 cm height, filled with 2000 g wet soil, more specifically 1600 g of dry soil with 25% water content. Bioremediation assays were all performed on stainless steel columns with 35.5 cm height, filled with 1420 g of wet soil, 1136 g dry soil with 25% water content, as well. The contamination was a mixture of benzene, toluene, ethylbenzene and ortho-xylene (25% of each), commonly called BTEoX. Also, the contamination level considered for the assays was 200 mg of contaminant for each kilogram of soil. The soil moisture was obtained by adding the microbial culture for the inoculated assays and sterilized distilled and demineralized water to the control assays.

For the assay 1, I1 second transfer was used to inoculate two of the four columns: Bioventing with saline soil (Bvs1) and bioremediation with saline soil (Bs1). In case of assay 2, was used I12, I13, I14 to inoculate four columns: Bioventing with saline soil (Bvs2), Bioventing with non-salted soil (Bv2), bioremediation with saline soil (Bs2) and bioremediation with non-salted soil (B2).

Prior to start, steel columns were well washed with water, soap and, in the end, with ethanol 96% inside. In the case of bioventing columns, all the entries were closed with septum, except for the air inlet and outlet that were linked to the air passage tubes. In the case of bioremediation, all the entries were closed with septums. Then, introduced the soil, previously moistened near to Bunsen burner, and columns were covered and closed. The contamination was made with the columns standing on the horizontal and injected equally on the entries S/S1 and E/E2.

After that, the columns were inverted three times, vertically, in order to promote homogeneity and better dispersion of contaminant. Finally, the columns were placed inside the thermostatic chamber at the temperature of 28°C.

Bioventing columns were submitted to daily ventilation during 15 minutes with an air flow of 20 ml/min. The air was introduced at the bottom of the columns and went out from the top to the flowmeter, passing to the respirometer, where the analysis of oxygen and carbon dioxide percentage were made. At the passage from the columns to the flowmeter was a valve that allowed to cut the air passage whenever needed. Also, in the system air inlet was another valve and a cellulose acetate filter, with pores of 0,20 μm to prevent the entrance of non-desirable particles (Carvalho, 2014).

The concentrations in the headspace were monitored daily by gas chromatography from an air sample of 50 μl , collected from the lateral entry (S/S1). In case of the bioventing assays, the monitoring was performed before pumping the air into the columns.

The assays were considered complete when the concentrations were below 0.5 mg/l three days in a row. Figure 3.10 illustrates the installation of the assays.



Figure 3.10: Bioremediation and Bioventilation assays setup

3.2.7 Analysis of the contaminants in the gas phase

The analysis of the contaminants in the gas phase was performed using gas chromatography, with a flame ionization detector (FID). The inlet gases were hydrogen and reconstituted air for the detector and nitrogen and helium as carrier gas, with a flow of 0.8 ml/min.

The chromatography analysis for the contaminants ran with the detector and the injector at the temperature of 260°C in splitless mode and column at the temperature of 55°C, in order to ensure a good resolution and segregation of the peaks.

During the experimental work, calibration curves were designated to cover the wide range of concentrations obtained on the assays. The method used for the calibration consisted in using patterns in Erlenmeyer flask previously weighted with distillate and demineralized water, in order to determinate its total volume, considering the density of water. Each pattern contained a known volume of contaminant, BTEoX, which allowed to determinate the respective concentration, considering the recipient volume, density of each component of the mixture and the volume of contaminant. The calibration curve contained individual curves for each component of the mixture (25% each): benzene, toluene, ethylbenzene and ortho-xylene. The contaminant was dropped inside the flask and left to volatilize. After that, it was made a chromatography analysis to discover the area of each component. The respective area could determinate, with the knowledge of the calibration curve, the respective concentration. Duplicate analyzes were performed with an error less than 10%.

The concentration of the contaminants on the patterns was calculated using equation 3.5:

$$C_p = \frac{V_c \times \rho_c}{V_f} \quad (3.5)$$

where C_p is the pattern concentration of the each contaminant, V_c is the volume of each contaminant, ρ_c is the contaminant density and, finally, V_f is the total volume of the Erlenmeyer flask.

3.2.8 Study of the distribution of the contaminants - Sorption Assays

In soil bioremediation studies, it is essential to evaluate the sorption impact on the contaminants and how they are distributed in the different phases of the soil, since sorption is a retardation mechanism that affects the mobility of contaminants.

The study of the contaminants distribution was performed using isotherms at the temperature of 28°C. The procedure to study the partition consisted in placing 700 g of wet soil (525 g of dry soil with 25% water content) inside of 1100 ml Erlenmeyer flask (Fig 3.11). Each flask was submitted to three cycles of sterilization. Then, it was administrated the BTEoX mixture inside each flask with different concentration, as it can be verified in table 3.5.

Table 3.5: Summary of the quantities used on the sorption assays

Volume of contaminant (μ l)	0.4	2	5	15	30	45	75	125	175
Mass of contaminant (mg)	0.35	1.74	3.47	13.02	26.04	39.05	65.09	108.48	151.87

After each administration, each flask rested for 7 days inside of a thermostatic chamber, allowing the contaminants to distribute in the different phases and reach the equilibrium. The monitoring was made after the seventh day, during three days in a row. If the concentrations were stabilized, then the flask could be dosed with new concentrations. The soil used was a saline residual granitic soil (SR) and a non-salted residual granitic soil (SRs), making it possible to compare the salt effect on the adsorption process.



Figure 3.11: Erlenmeyer flask for sorption assays

The isotherms were graphically represented considering the contaminant concentration in the gas phase depending on the contaminant concentration on the soil. The determination of the concentration of the contaminants in the different phases were made with the help of equations 3.6 to 3.11:

$$C_{aq} = \frac{C_{gas}}{H} \quad (3.6)$$

$$M_{aq} = C_{aq} \times V_{aq} \quad (3.7)$$

$$M_{gas} = C_{gas} \times V_{gas} \quad (3.8)$$

$$M_s = M_t - M_{gas} - M_{aq} \quad (3.9)$$

$$C_s = \frac{M_s}{M_{soil}} \quad (3.10)$$

$$V_{gas} = V_t - V_w - V_{dp} \quad (3.11)$$

where the C_{aq} is the concentration of the contaminants (mg/l) in the aqueous phase, C_{gas} is the contaminant concentration (mg/l) in the gas phase, H is the Henry's constant at the temperature of the experience, M_{gas} is the mass of contaminant (mg) in the gas phase, M_{aq} is the mass of contaminant (mg) in the aqueous phase, V_{aq} is the contaminant volume (L) in the aqueous phase, V_{gas} is the contaminant volume (L) in the gas phase, M_s is the mass of contaminant (mg) in the solid phase, M_t is the total mass of contaminant (mg), M_{soil} is the total mass of dry soil (mg), C_s is the concentration of the contaminant (mg/Kg) in the solid phase, V_t is the total volume, V_w is the volume of water and V_{dp} is the volume of the dry particles.

As mentioned in 2.7.2, the Langmuir and Freundlich models are very useful to understand the adsorption process and estimate the amount of contaminants that is adsorbed by the soil. After the chromatographic analysis, this relation was obtained using the equation 12 and 13:

$$C_s = K_f \cdot C_{gas}^n \quad (3.12)$$

$$C_s = \frac{K_L \cdot b \cdot C_{gas}}{1 + K_L \cdot C_{gas}} \quad (3.13)$$

where C_s is the amount of contaminant (mg/kg), K_f is the equilibrium constant of Freundlich, C_{gas} is the concentration of the contaminant (mg/l), n is the affinity between contaminant and the solid phase, K_L is a equilibrium of Langmuir b is the maximum capacity of adsorption

The methodology of the study of the contaminants partition used was adapted from the doctoral thesis of Carvalho, 2014.

4

Results and Discussion

4.1 Characterization of the soil

For the complete understanding of the soil remediation, it becomes important to acquire the knowledge of the soil characteristics. Therefore, the soil was subjected to geotechnical, physical and chemical characterization.

The particle size distribution obtained by sieving test and by granulometer analysis is represented in the figure 4.1.

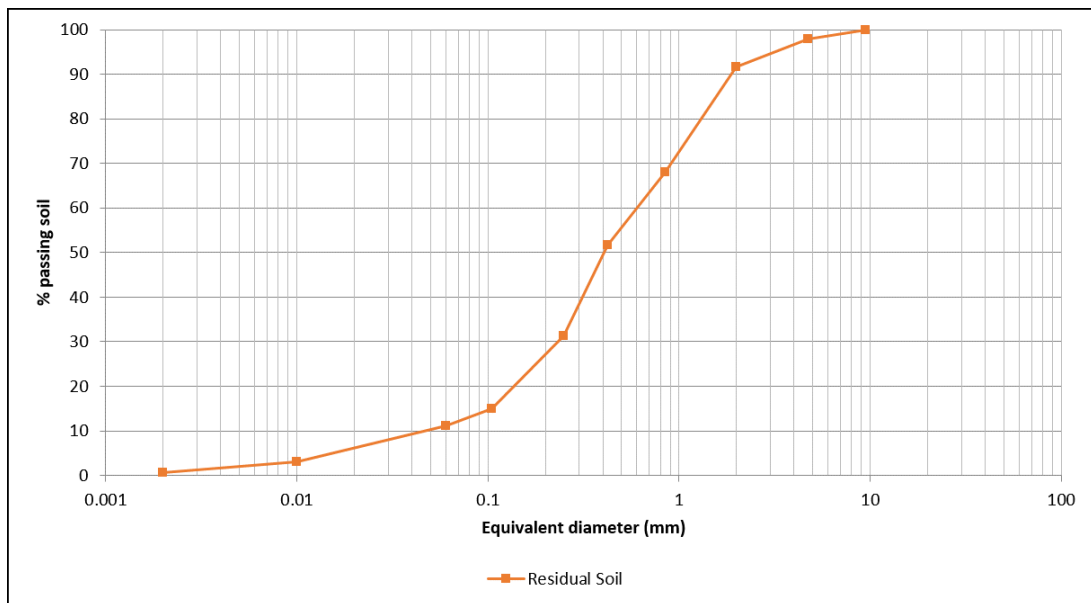


Figure 4.1: Particle size distribution.

The granulometric analysis showed that the granitic soil had an extensive particle size and was well graded. Also, the analysis revealed that the soil was predominantly sandy (81%), with 10% silt, 8% gravel and 1% clay, as represented in the table 4.1.

Table 4.1: Geotechnical characteristics of the residual granitic soil

Residual granitic soil (SR)	
Gravel	8%
Sand	81%
Silt	10%
Clay	1%

According to the Feret diagram (Fig. 4.2), the granulometric classification of the residual granitic soil is a was loamy sand. The classification was taking into account the obtained values of the percentage of sand, silt and clay. The crossing of the three values in the diagram represents the designation assigned to the type of soil.

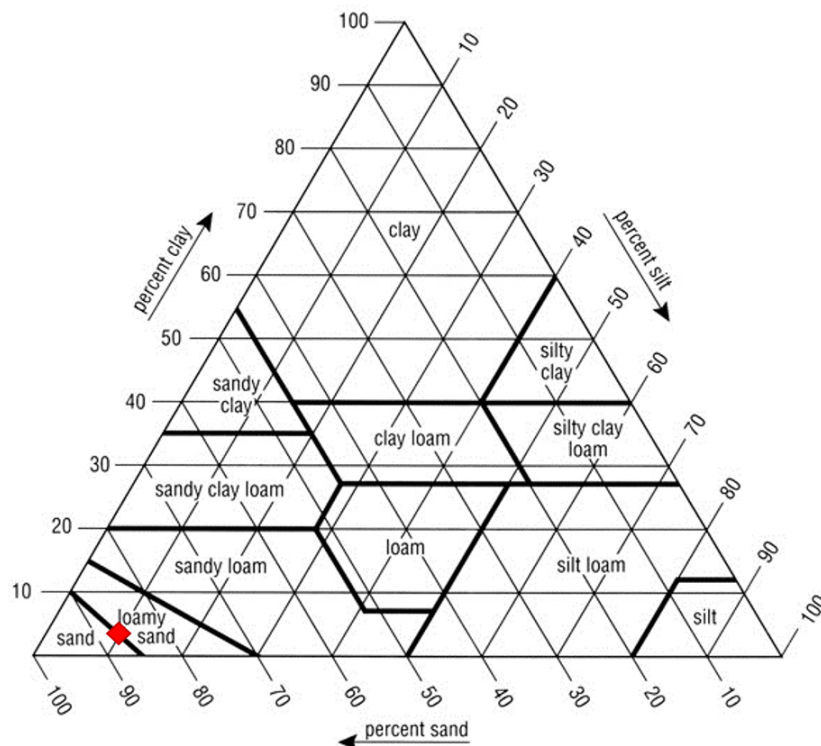


Figure 4.2: Representation of the residual granitic soil at the Feret diagram.

The physical and chemical parameters obtained are presented in the tables 4.2 and 4.3.

Table 4.2: Physical and chemical parameters of the soil

Bulk density (ρ)	947 kg/m ³	Organic matter (MO)	1.88%
Total organic Carbon	<Detection Limit	Water saturation content (Ws)	51.5%
Total Carbon		Cationic exchange capacity	4.50x10 ⁻⁶ mol/Kg (SRs)
			0.2 mol/Kg (SR)
Inorganic Carbon		Conductivity	920 μ S/cm (SRs)
			38.8 μ S/cm (SR)

Table 4.3: Values for the pH according to the ISEP-IPP laboratories method and *Carvalho, 2014.

		H₂O	KCl	CaCl₂
pH	15 minutes	3.85	-	-
	1h15	4.01	-	-
	2h20	4.03	3.52	3.85
	20 minutes	4.5*	-	-

The measured parameters allow to characterize the soil as having low content of organic matter (1.88%) and very low content of organic and inorganic carbon, with values below the detection limit. This soil was obtained about two to three meters deep, so the values make sense, since the organic matter is minor, in relation to the surface. Also, these parameters might suggest that the existing organic matter did not have a great effect on sorption of the contaminants.

Saturation water content is 51.5%. This value allowed to determine the adopted moisture for the trials, in order to ensure the ideal moisture in the microorganism environment. Also, it allowed to prevent the saturation of the soil, promoting the passage of the air through the porous, in the bioventing assays (Carvalho, 2014).

Table 4.3 presents the pH values for the ISEP-IPP method, at 15 minutes, 1h:15min and 2h:20 min in H₂O. The reading of the pH in KCl and CaCl₂ was only made after two hours. As for the Carvalho (2014), the reading was made after 20 minutes resting. In both methods, the pH suggests a slightly acid soil.

Table 4.4 refers to the elemental chemical composition of the soil, in percentage.

Table 4.4: X-Ray fluorescence results for the elemental chemical composition

K	Ca	Ti	V	Mn	Fe	Co	Ni	Zn
0.288%	0.047%	0.243%	0.002%	0.112%	0.001%	0.001%	0.002%	0%
Rb	Sr	Y	Zr	Nb	Mo	Cd	W	U
0.020%	0%	0%	0%	0%	0%	0%	0%	0%

4.2 Quantification of the biomass

Quantification of the biomass in remediation tests are important, since it gives direct information about the development of the consortium in the environment conditions. A comparative quantification of the biomass, before and after the performance of the remediation tests, is illustrated in figure 4.3.

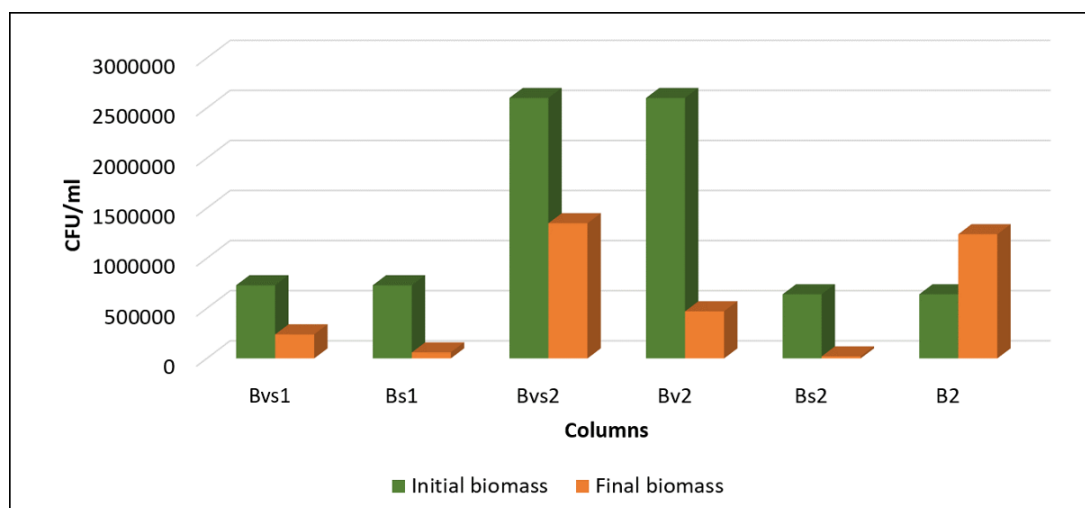


Figure 4.3: Quantification of the biomass

The graphic displays a general biomass decrease after the performance of the bioventing and bioremediation tests, with the exception of B2 test.

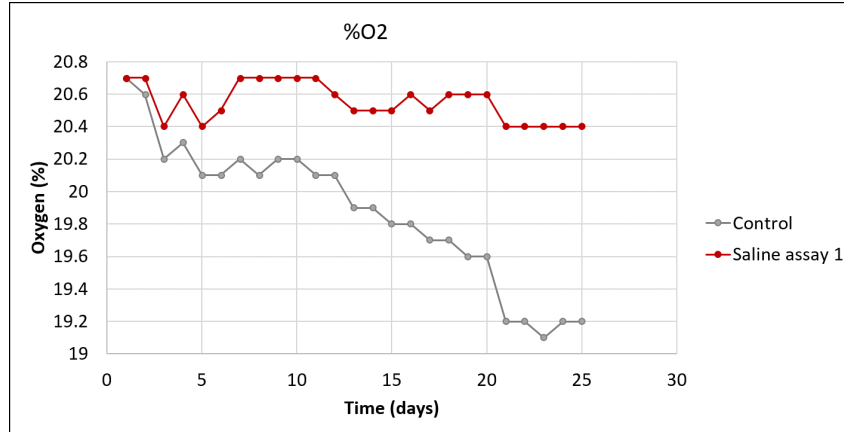
First assay, Bv1 e Bs1, showed an almost total biomass reduction inside the columns. Regarding the second bioventing test (Bv2), the entirely death of the consortium was not verified, despite having a great decrease of the total population. In case of the saline bioventing (Bvs2) the decrease observed was not so accentuated, which can indicated the existence of biological activity, yet just enough to keep the population survival.

Finally, concerning the non-salted bioremediation assay, Bs2, the total death of the microorganism was verified, however, in the non-salted column, the microbial population, prospered and increased.

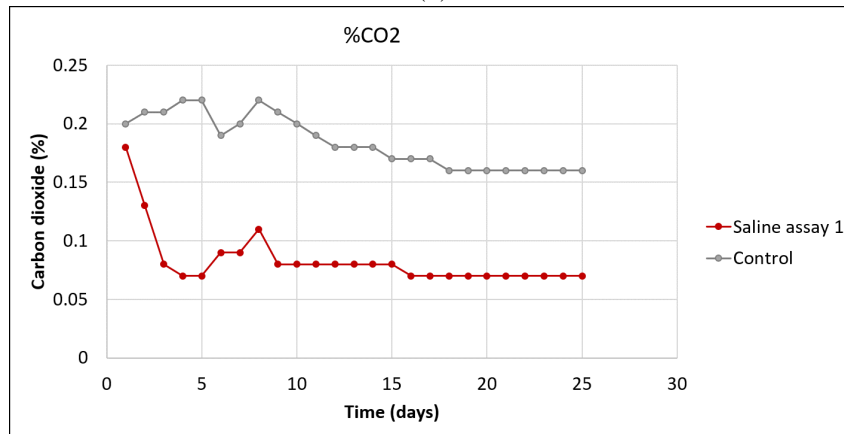
4.3 Analysis of the respirometry

Respirometry is capable of measuring the amount of oxygen used and carbon dioxide produced by the microorganisms, displaying a curve that can translate the consortium metabolism. In this sense, this technology had a great importance on the understanding of the consortium biological activity during the experimental work.

Figure 4.4 presents the respirometric results of the first bioventing assay.



(a)

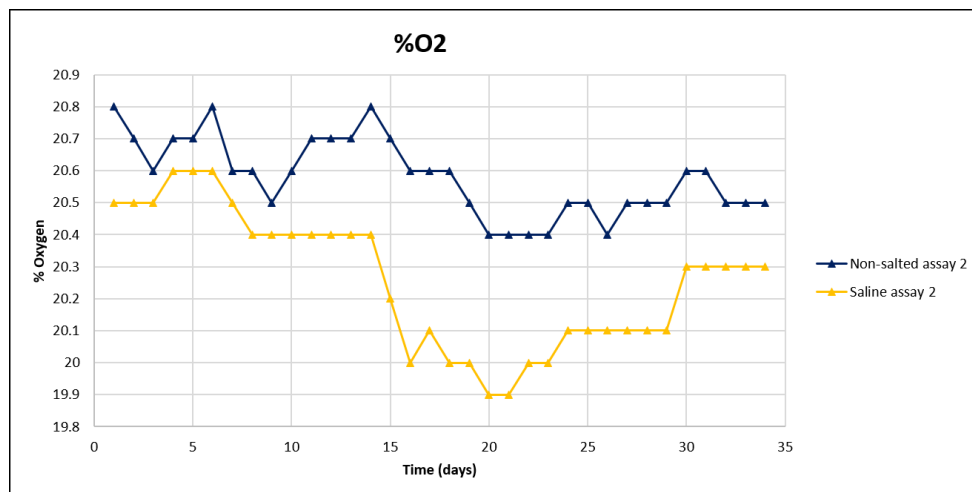


(b)

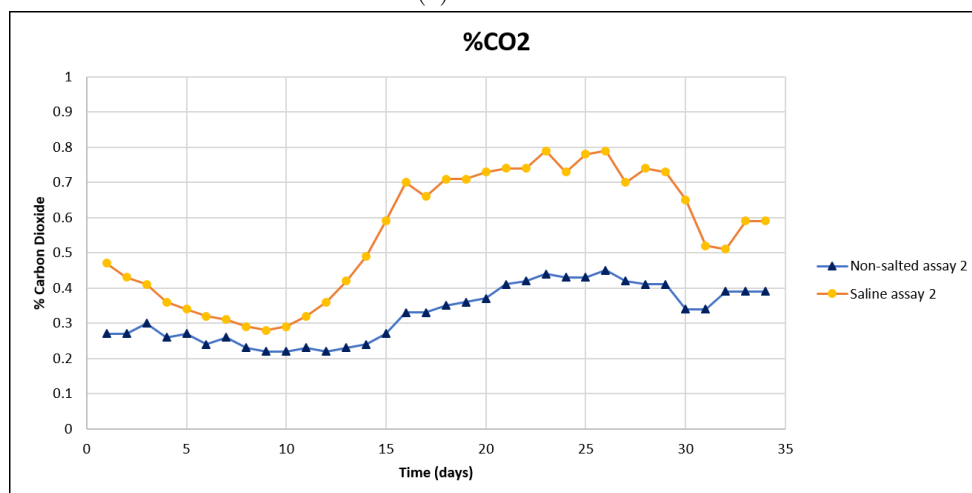
Figure 4.4: Respirometry results for the first assay. (a) Oxygen. (b) Carbon dioxide.

Concerning the first saline assay, it was not verified significant evidences of metabolic activity. In the early days, until day 5 (figure 4.4b), it can be verified a decrease of the carbon dioxide concentration, that reached a minimum value. After this, it could be noticed a slightly concentration increase, but eventually the concentration stabilized until the end of the test. The horizontal profile corresponds to a non production of CO_2 , evidencing that there were not degradation of contaminants. In case of the oxygen, figure 4.4a, there were small oscillations on the concentration, however it was negligible. So, both indicators suggested that there were no signs of relevant microbial activity, since the beginning of the experimental work, which might be due to the saline environment and contamination levels.

On the other hand, the control test presented a continuous drop of the oxygen concentration, indicating an activity that was not possible to explain, despite being a sterilized and uninoculated soil. Nevertheless, the carbon dioxide concentration kept constant values during the experiment, as expected.



(a)



(b)

Figure 4.5: Respirometry results for the second assay. (a) Oxygen. (b) Carbon dioxide.

In the second assay, figure 4.5, more significant biological activity was verified. For the saline assay, figure 4.5a, respirometry revealed a considerable drop on the oxygen concentration, until day 20, demonstrating signs of biological activity. This activity was considered low, since the different between the oxygen initial and final values were only 0.6%. In fact, the final biomass quantification inside the column showed about half the initial population, which could evidence a minimal activity in order to keep the consortium survival.

In addition, the carbon dioxide concentration varied in accordance with the use of oxygen. However, until day seven, there was a decrease of the carbon dioxide that reached a minimum value that increased until day 23. This trend is also manifested in the first saline assay, which

seemed to be a salt mark by the consortium.

Regarding the non-salted assay, a low microbial activity was observed, presenting a variation of the oxygen and carbon dioxide concentration in a small range of values: O_2 had a variation of 0.4% and the CO_2 had a variation of about 0.15% which was not quite relevant.

Figure 4.6 aims to compare the respirometric results for both trials.

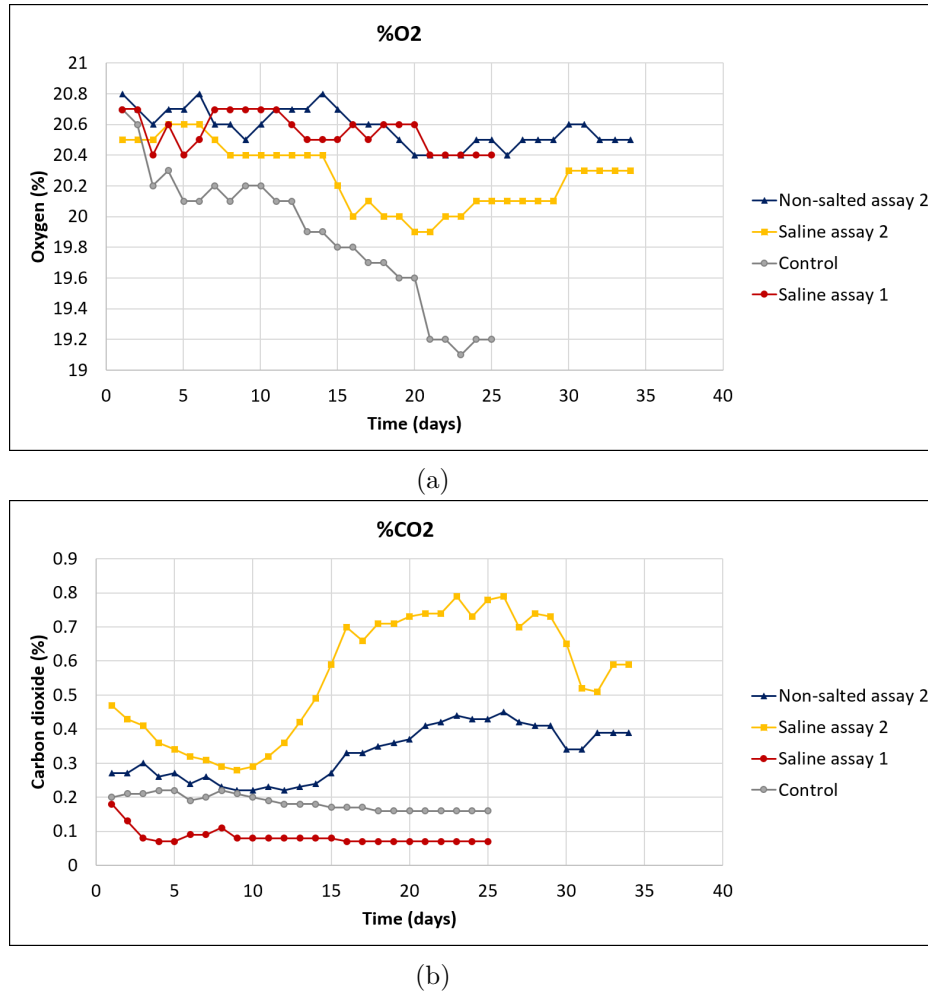


Figure 4.6: Comparison of the respirometric results for both assays. (a) Oxygen. (b) Carbon dioxide.

A global and comparative view of the respirometric results showed that there was an insignificant consumption of oxygen and carbon dioxide production, which revealed low consumption of the organic compounds inside the columns. Besides, second assay presented a low biological activity. Regarding the first assay, it was considered that the consortium can not resist the environmental conditions.

Previous studies, such as Carvalho PhD thesis, in 2014, used the same sample and obtained an extremely significant activity and degradation of the contaminants, which leads to the conclusion that the consortium used may have lost some capacity to degrade and to resist high contaminations levels, with time, and also can not tolerate the soil salinity.

4.4 Analysis of remediation assays

4.4.1 Bioventing

First Assay (A1)

Bioventing results, regarding the first assay, are presented in figure 4.7.

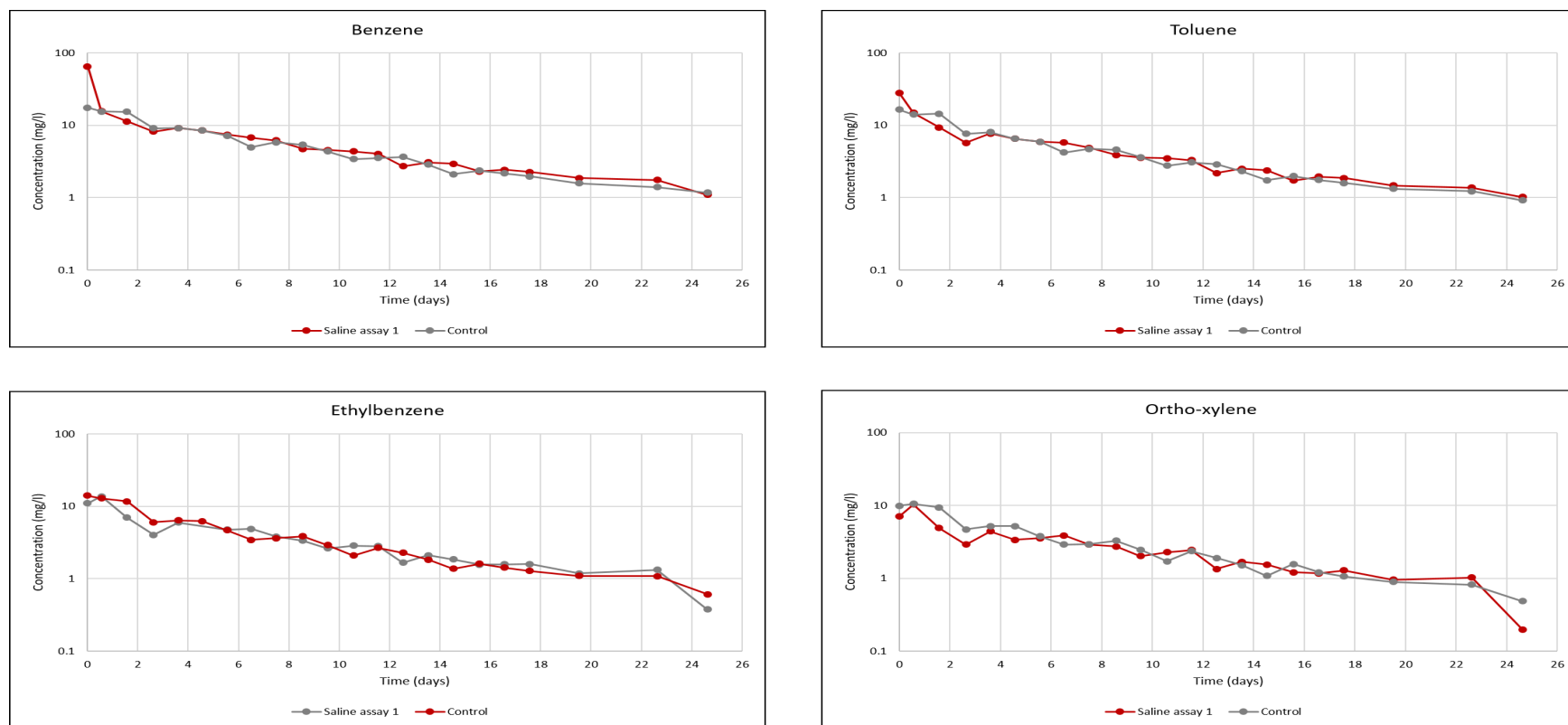


Figure 4.7: Results from the bioventing columns regarding the first assay

Second Assay (A2)

The bioventing results for the second test are present in the figure 4.8.

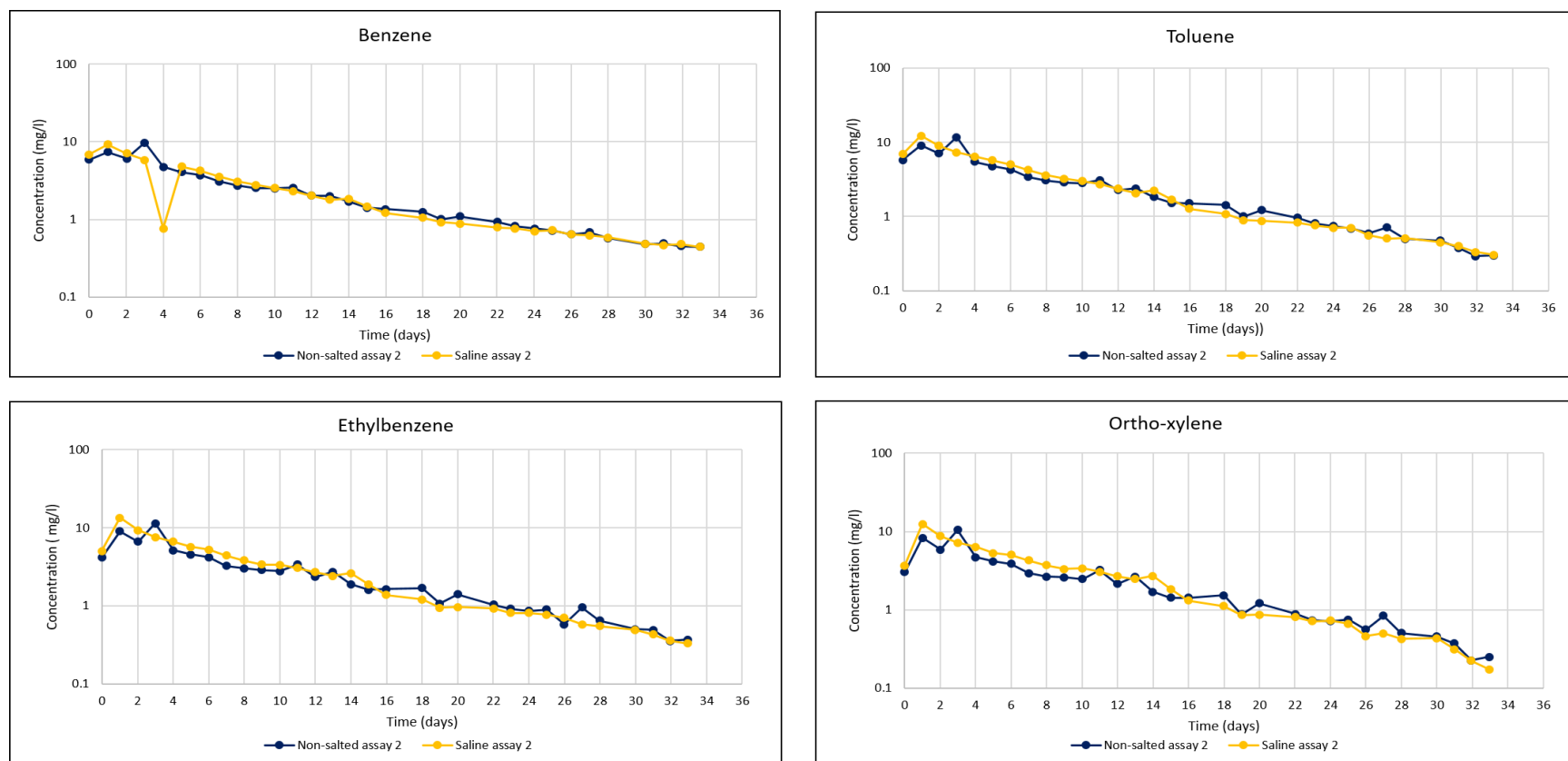


Figure 4.8: Results from the bioventing columns regarding the second assay

Comparative analysis of first and second assays (A1 vs A2)

A comparative analysis of first and second assays are illustrated in figure 4.9.

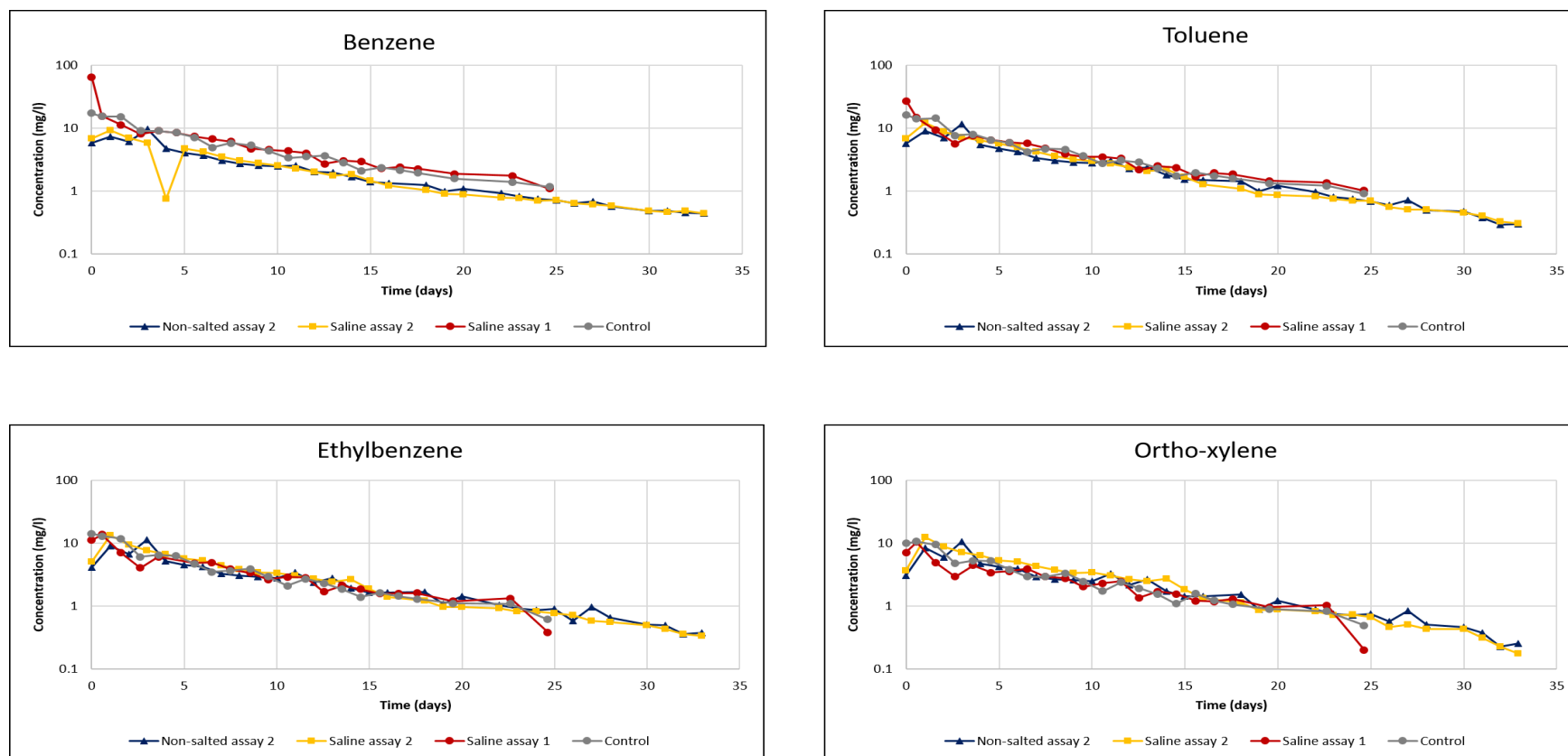


Figure 4.9: Comparison of the results from the bioventing columns

The graphics of figure 4.7, concerning the first bioventing assay presents the concentration values as a function of the experience time, in days. The results showed that the control and saline soil columns were overlapping, presenting minimal differences between each other. In fact, this is in agreement with the results from the respirometry, where it can be concluded that the microorganisms did not degraded the contaminants. The concentration decrease that was verified in the assay was essentially due to the contaminants entrainment made by the air flow as a consequence of the daily ventilation.

The bioventing process promote the biodegradation by applying air flow through the column, however there is a natural volatilization of the pollutants. The tendency lines of each contaminant revealed that benzene had the largest slope, as it is the most volatile of the mixture, then comes the toluene and ethylbenzene. Ortho-xylene had the less pronounced slope, since it is the less volatile compound.

Concerning the second assay, in figure 4.8 was observed a similar behavior comparing the first test. The non-salted and saline columns were overlapping, which might suggest that the salinity does not induce any negative effect regarding the soil remediation.

On the other hand, making a global comparative view of the results, it was verified that the performed assays did not presented significant difference. Inoculated columns approached the control line, demonstrating inexistent degradation. Bioventing results suggested that the consortium was not capable of biodegraded the contaminants, with or without the saline conditions. In fact, it became difficult to discuss about the effect of the salt with the selected consortium on the bioventing test.

4.4.2 Bioremediation

First Assay (A1)

Bioremediation results for the first assay are illustrated in figure 4.10.

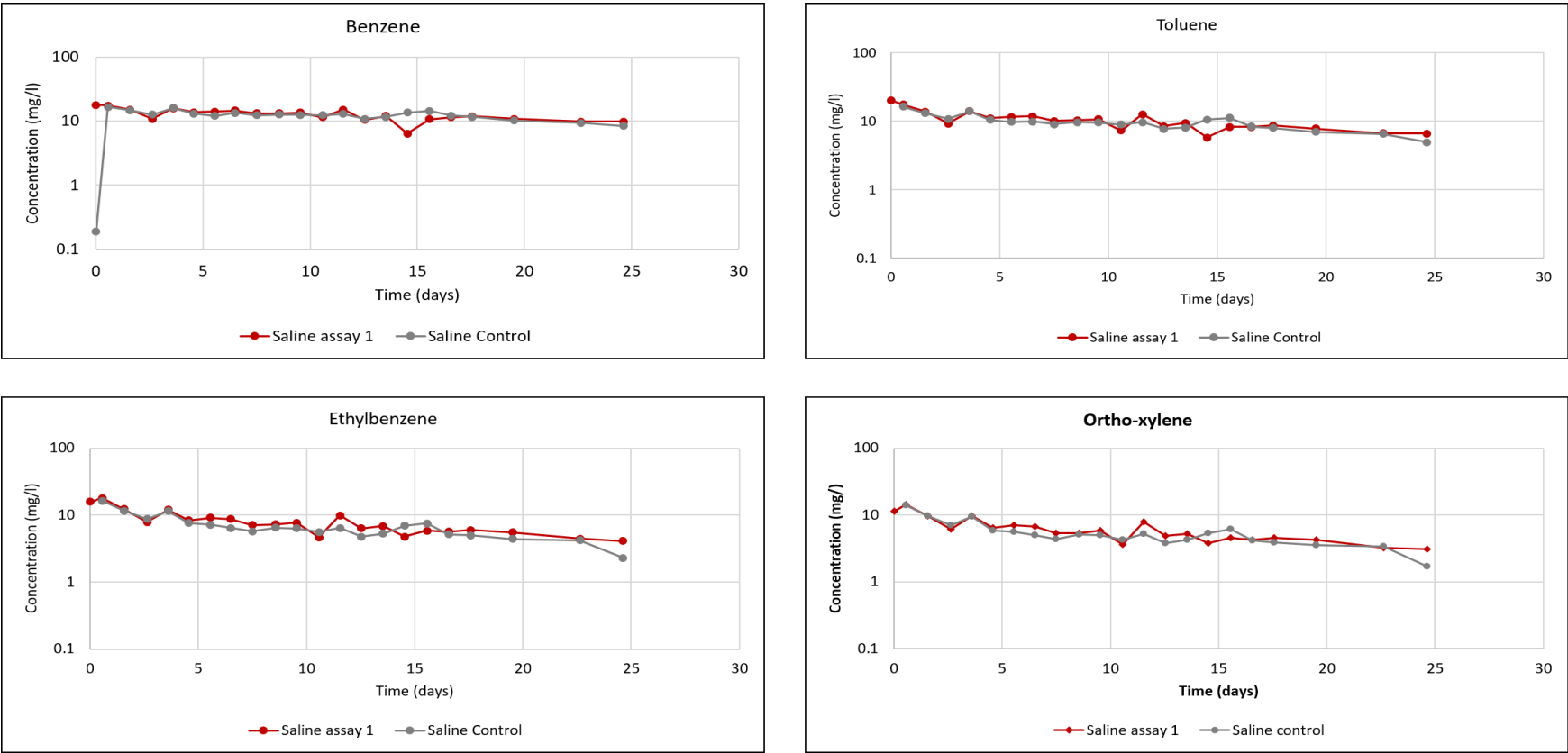


Figure 4.10: Results from the bioremediation columns regarding the first assay

Second Assay (A2)

Bioremediation columns results concerning the second assay are presented in figure 4.11.

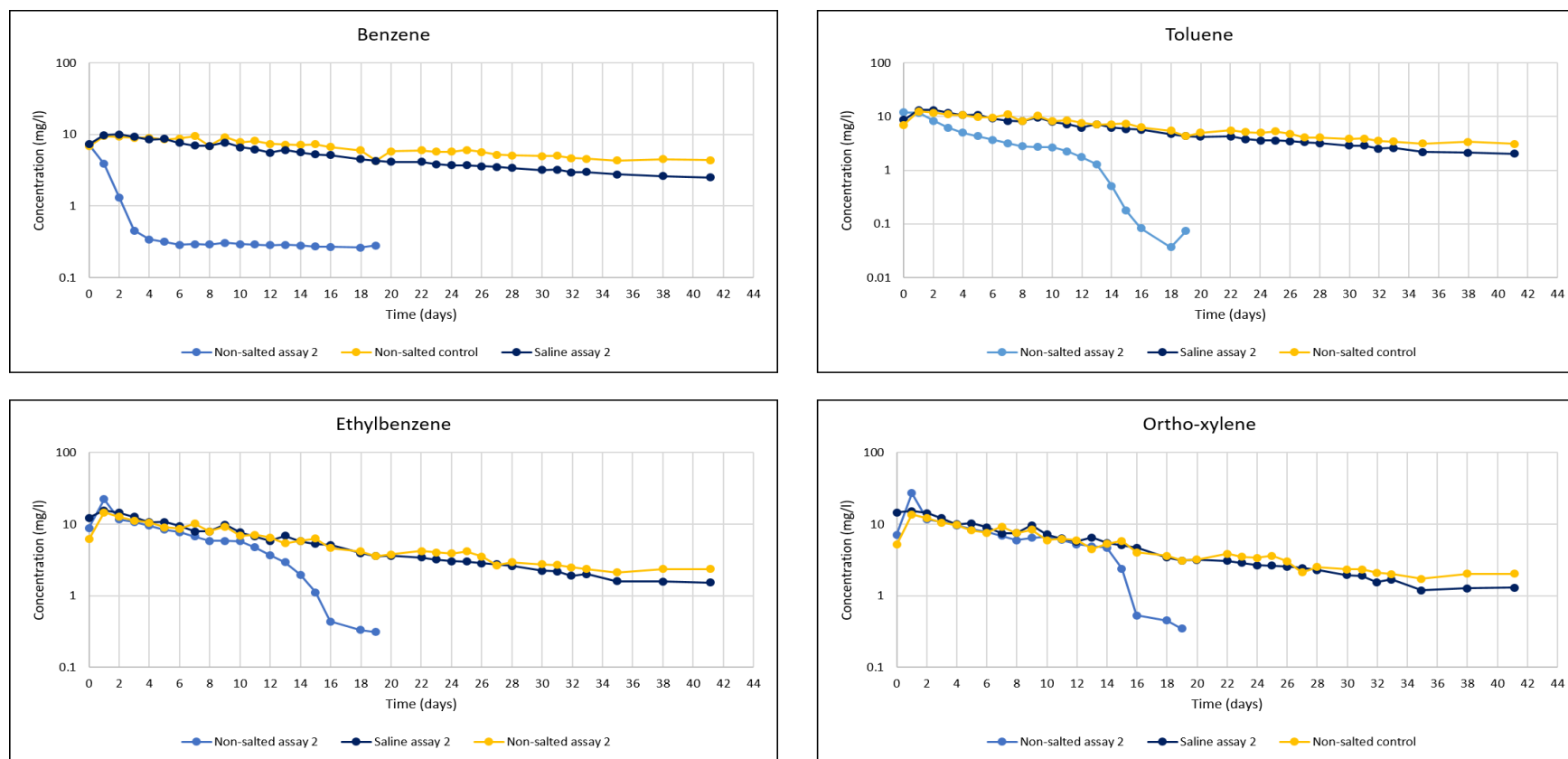


Figure 4.11: Results from the bioremediation columns regarding the second assay

Comparative analysis of first and second assays

A comparative analysis of the bioremediation results was made in order to have a global view of both assays. The comparison can be observed in figure 4.12.

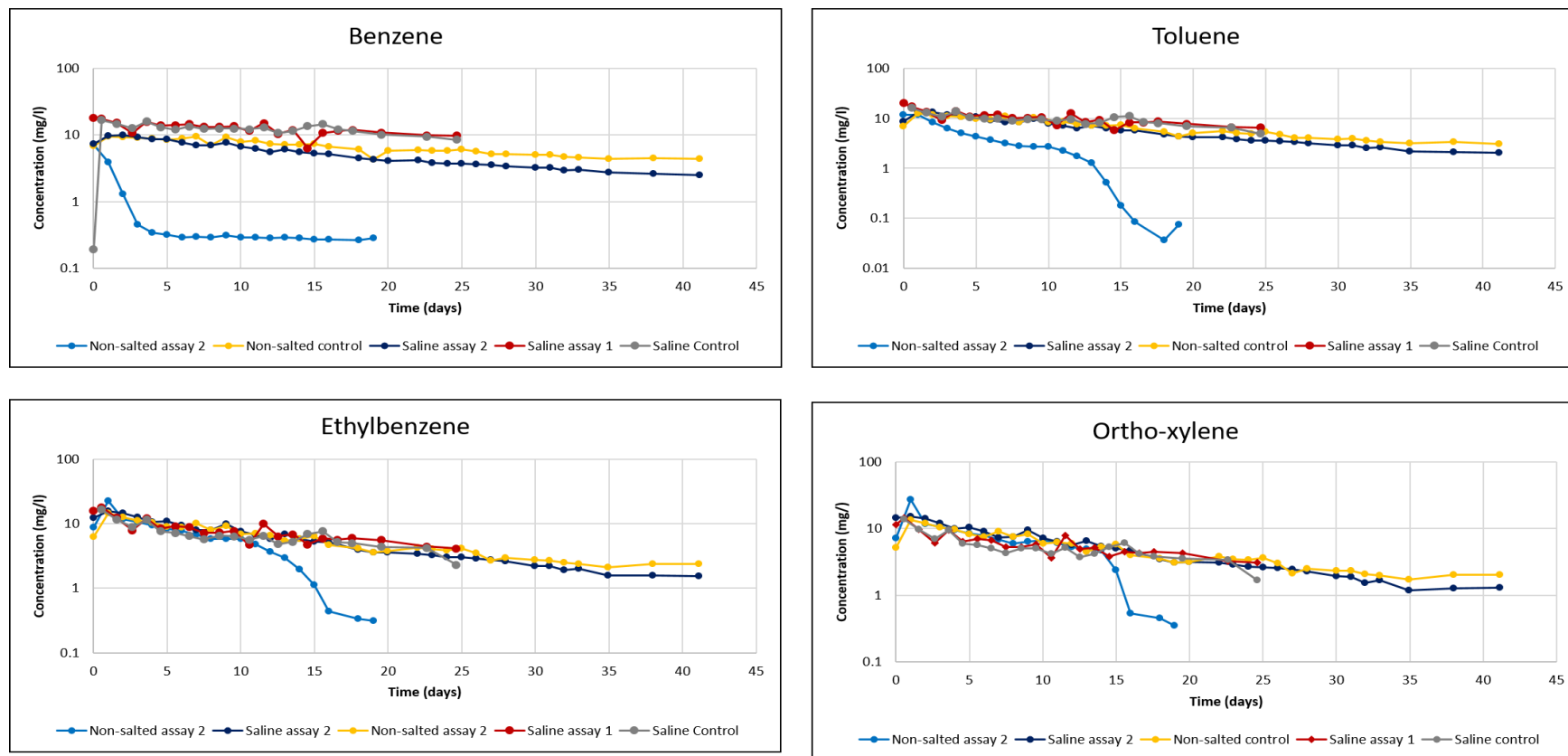


Figure 4.12: Comparison of the results from the bioremediation columns

Figure 4.10 showed an overlapping of both columns (inoculated saline and saline control). The overlapping means that there was no biodegradation. Despite this, it was observed, with more evidence, a decrease of the ethylbenzene and ortho-xylene concentration, which is due to the sorption mechanisms. Benzene and toluene, because are more volatile, are less adsorbed by the solid phase of the soil, contrary to what was observed with ethylbenzene and o-xylene that presented lower values of vapor pressure, being adsorbed with more intensity.

Concerning the non-salted column from the second assay (fig. 4.11), it was verified a very effective consumption of contaminants, within 20 days. The degradation of the pollutants was made by stages, leading to conclude that the consortium started to consume the chemically less complex elements, such as benzene. When benzene ran out, at the end of 4 to 5 days, the microorganisms started to degraded toluene. However, toluene concentration was already decreasing while benzene was being consumed, indicating a competition for carbon source. In addition, toluene was consumed with more intensity from day 10, which may indicate that, since the beginning, microorganisms have entered in an adaptation phase in order to be able to consume more easily the remaining contaminants. Moreover, the degradation of the more complex molecules, ethylbenzene and ortho-xylene, was made in parallel, since they are two similar molecules. Concentration decrease of these two contaminants, in the early days, were probably due to the adsorption mechanisms.

This preference by the microorganisms was probably related with the fact that the consortium, in the enrichment culture, grew with benzene as a carbon source. In this sense, the consortium gained more affinity to this molecule and less capability to decompose more complex substances.

Furthermore, it is important to mention some aspects about the non-salted column conditions. First, the soil inside the column was not sterilized, presenting evidences of autochthonous population. Secondly, the column had less volume of soil inside, which implied a bigger volume in the headspace, increasing the amount of oxygen available for the biological aerobic reactions. Finally, a fungus was found inside the column, as well as in the saline column, that was not revealed in the original enrichment culture.

In relation to the saline column, it was observed that the saline column got closer to both control lines (fig.4.12), evidencing no consumption of the pollutants. Considering that inside this column, was also found the same fungus from the non-salted assay, it is possible to conclude that the salinity in the soil induce negative effects on the biological activity, leading to the death of the microbial consortium.

Besides, only the non-salted assay showed an effective removal of the contaminants, that it is believe to be due to the existing fungus inside the column. Also, the same fungus, did not survive in the salt conditions, not making possible to have an effective soil remediation. The remaining columns were overlapping the control lines during the experimental work (fig.4.12), meaning that the biodegradation of the contaminants was not possible.

Comparative analysis of bioremediation and bioventing tests

Assay 1 (A1)

A comparative evaluation of bioventing and bioremediation assays is showed in the figure 4.13.

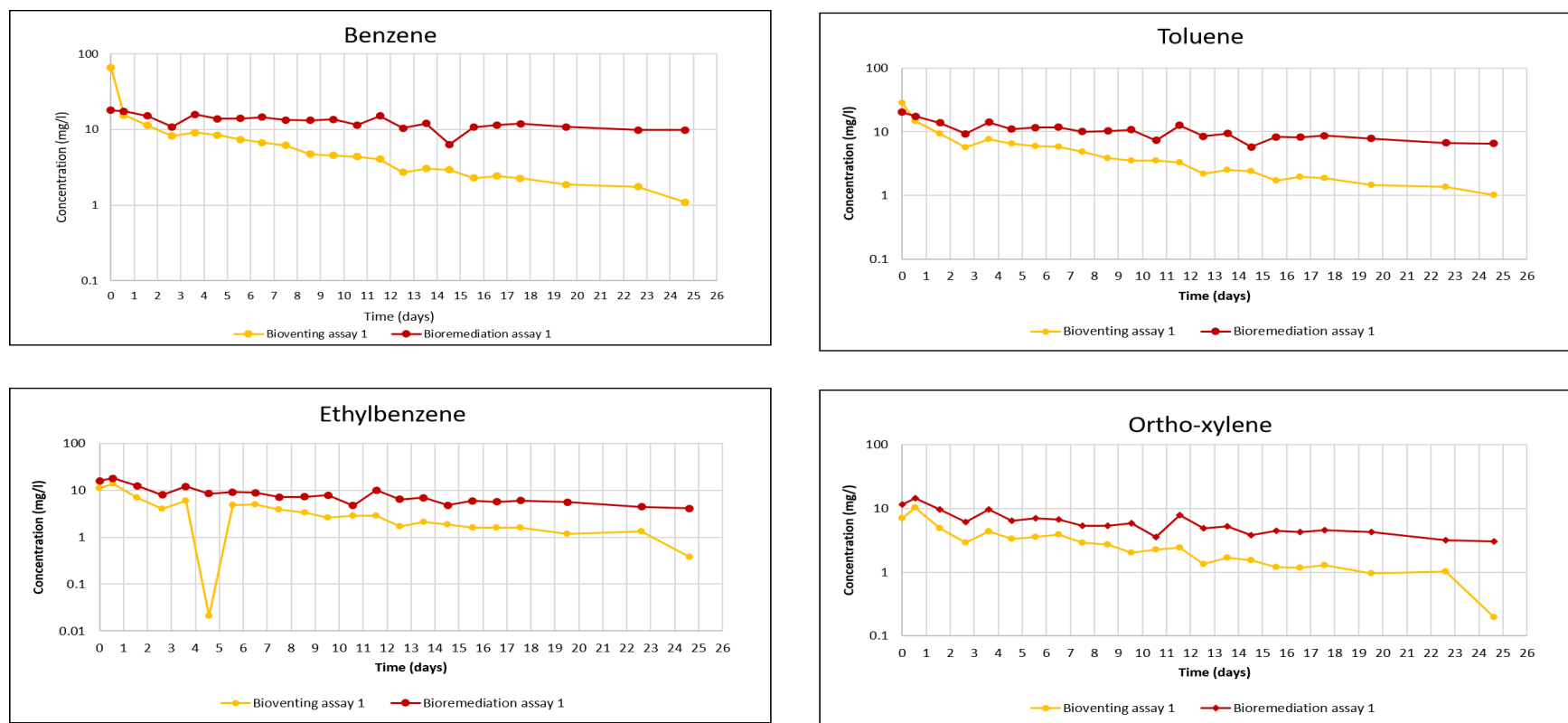


Figure 4.13: Comparative results of bioventing and bioremediation for benzene, toluene, ethylbenzene and ortho-xylene - assay 1

Assay 2 (A2)

Concerning the second trial, it is presented, in figure 4.14, a comparative analysis of bioventing and bioremediation assays.

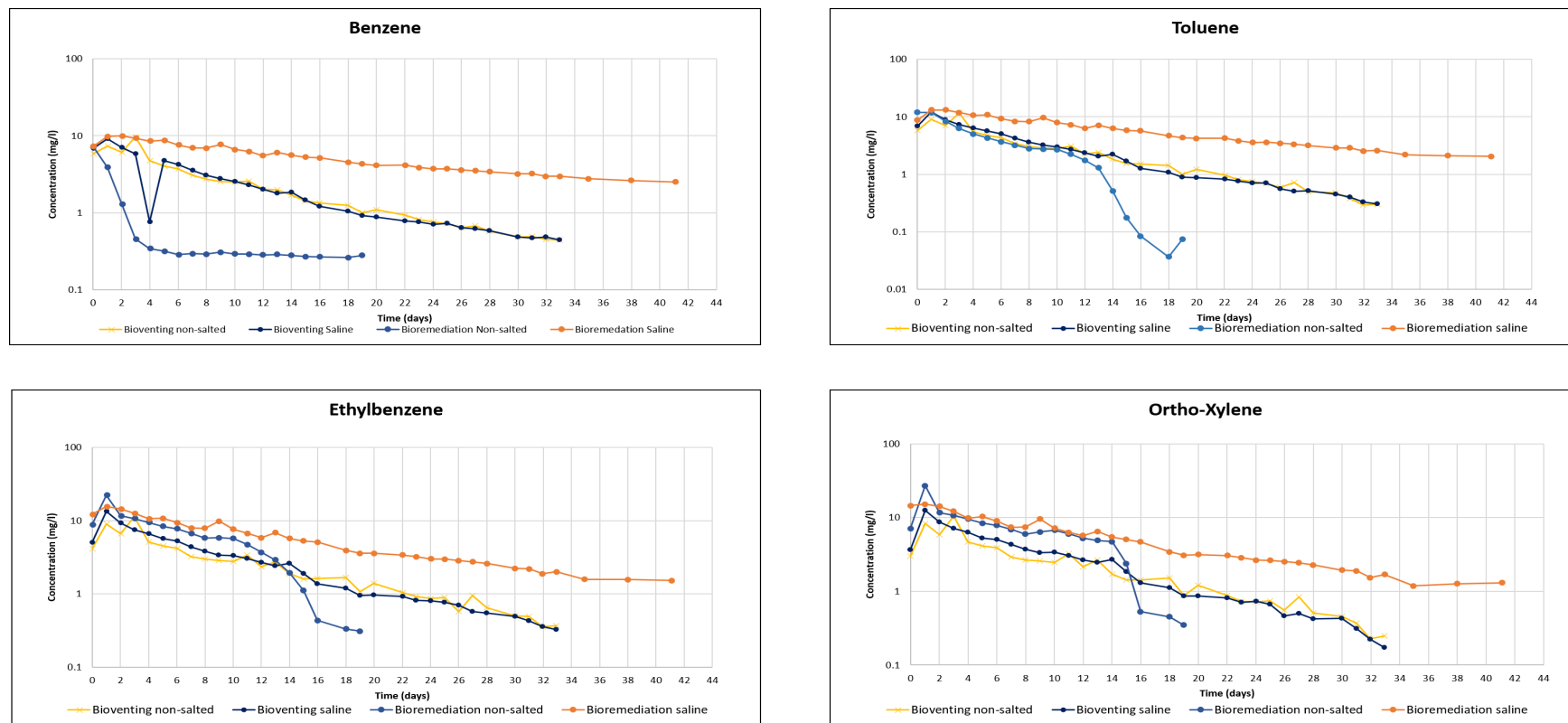


Figure 4.14: Comparative results of bioventing and bioremediation for benzene, toluene, ethylbenzene and ortho-xylene - assay 2

In both assays, the remediation of the soil was not effective, except for the non-salted column, in assay 2. The difference between the bioremediation and bioventing lines corresponds essentially to the contaminants volatilization as a consequence of the daily ventilation. Also, the decrease of the concentration in the bioremediation assay (excepting for the non-salted column) are a consequence of adsorption mechanisms.

In addition, the contaminants properties could be well evidenced in both figures. Benzene is the most volatile compound of the mixture, because has a higher value of vapor pressure, so presented a larger slope. On contrary, ortho-xylene has a lower vapor pressure value, so the volatilization was less verified. Sorption was observed with more intensity in less volatile chemical elements. In bioremediation assays, ethylbenzene and ortho-xylene presented a higher slope, since they were more adsorbed by soil.

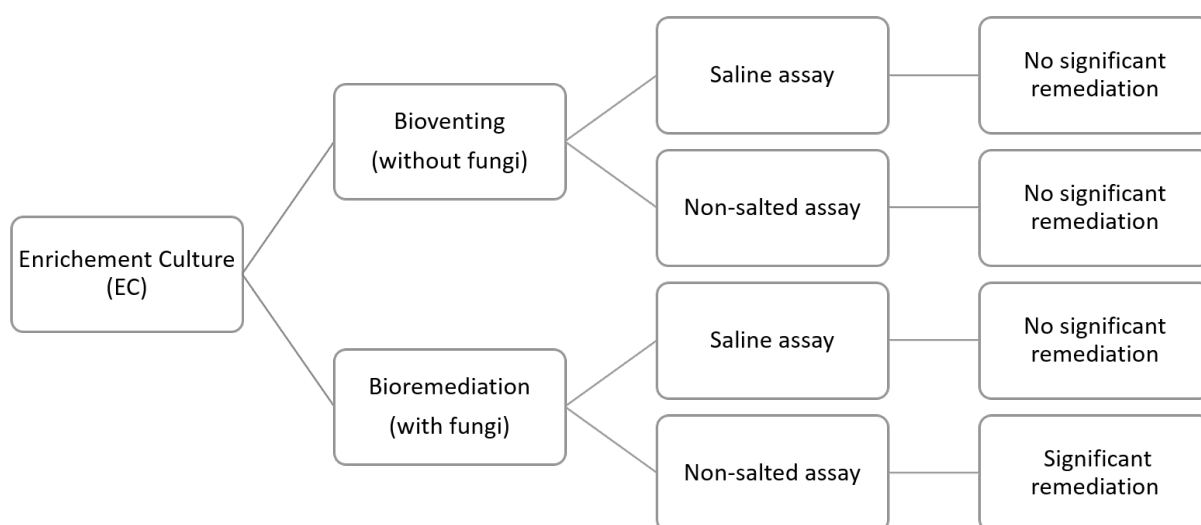
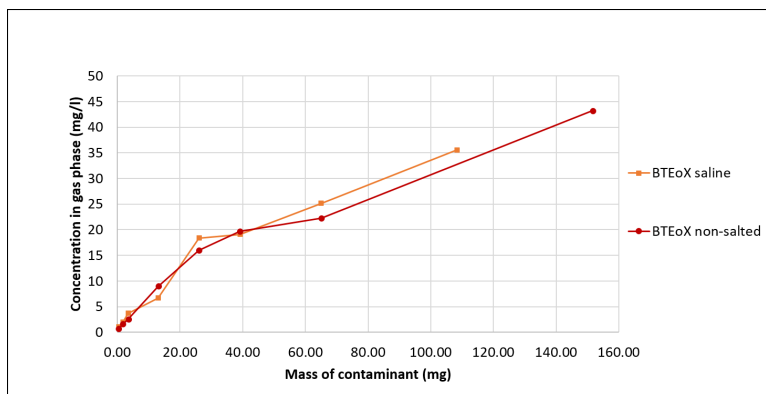


Figure 4.15: Summary of the remediation tests

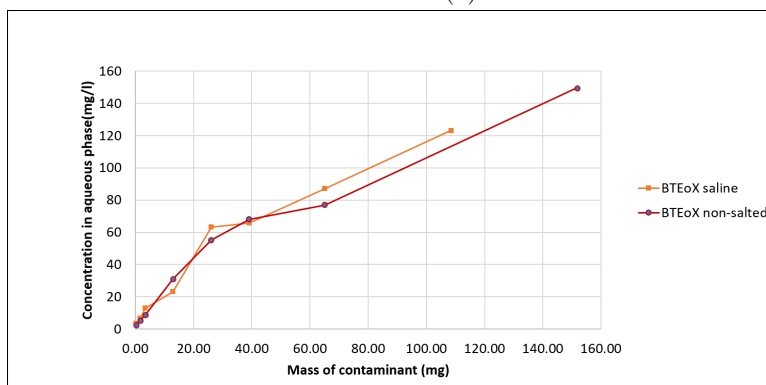
At the end of the assays, it was verified, in bioremediation tests, that there was an effective remediation of the soil, regarding the non-salted assay, probably due to the existence of a fungus. The same fungus did not present remediation capability in a saline environment. Bioventing tests without fungi did not present signs of soil bioremediation.

4.5 Analysis of the distribution of the contaminants

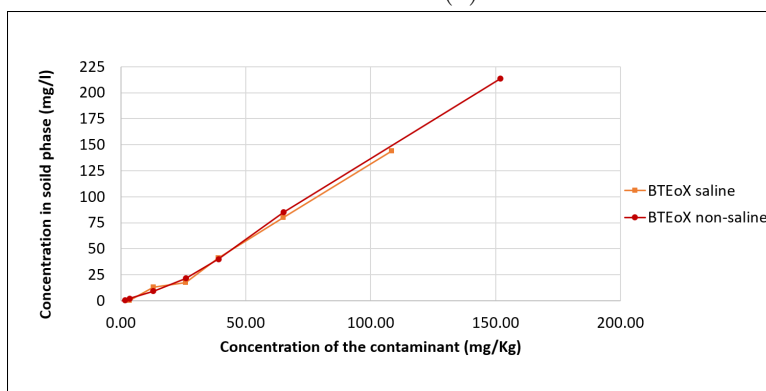
The results of the contaminants distribution are presented in figure 4.16. It is important to mention that, two concentrations were not considered, due to possible leakage in the flasks. In saline assay, the volume of 175 μl was not considered and 125 μl in non-salted assay.



(a)



(b)



(c)

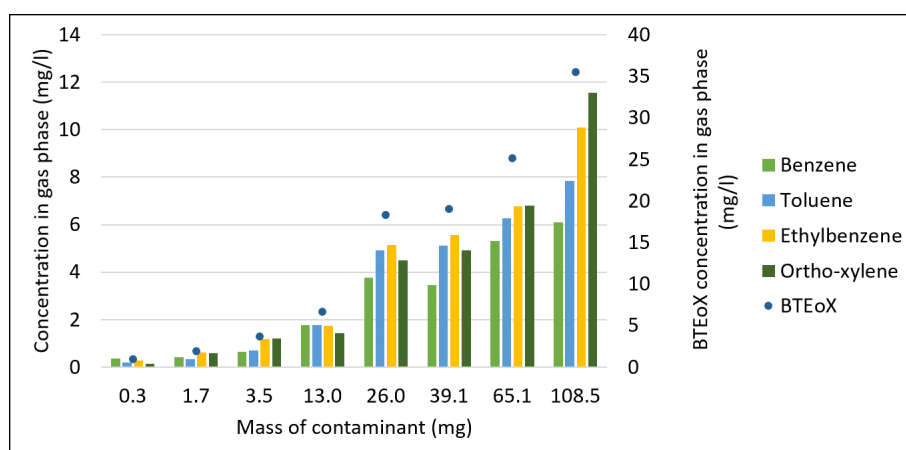
Figure 4.16: Distribution of the contaminants in different phases. (a) Gas phase, (b) Aqueous phase, (c) Solid phase.

Concerning the figure 4.16a and 4.16b, it was verified that both assays are very similar to each other, however near 60-70 mg it was noticed a slightly separation between the two assays. Also, the concentrations increased when was administrated more contaminant into the flask, despite of having a stabilization stage from 20-40 mg of contaminant in case of the saline assay and from 40-60 mg of contaminant, for non-salted assay. It could be evidenced three different phases: a first phase where the concentration had a exponential growth. The second phase was related to the concentrations stabilization. Finally, a third phase where the mass of contaminant increased linearly in the gas and aqueous phase.

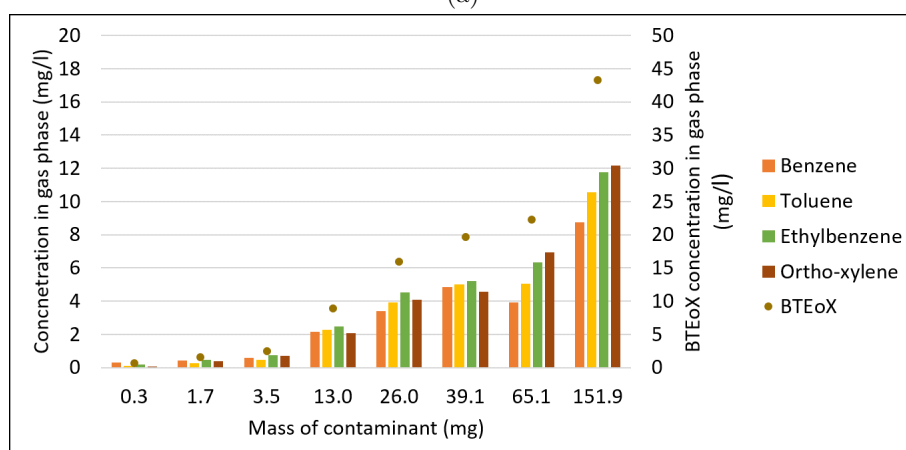
Regarding the aqueous phase, a similar behavior was verified, however the total mass in this phase was considerably larger, which could be related with phenomena of dissolution.

In relation to the solid phase, an exponential growth was noticed, in both assays, suggesting availability of free spaces in the soil to adsorb.

A comparative analysis of the distribution of the individual contaminants and the mixture, regarding the gas phase, for both assays, is represented in the figure 4.17.



(a)



(b)

Figure 4.17: Comparison of the respirometric for both assays. (a) Saline Assay. (b) Non-salted assay.

The individual distribution, for both cases, showed that when the flask contained a small

of mass (0.3 mg), the concentration of benzene was larger in the gas phase, compared with the other compounds. Nevertheless, during the addition of mass, it was verified a tendency for the less volatile compounds to have a larger concentration in the gas phase, despite the expectation that benzene and toluene, because they are more volatile, would have greater presence in this phase. In fact, given the physical and chemical properties of these contaminants, there might have been phenomena of dissolution among them, influencing the distribution that should have been if they were given alone.

The determination of the concentrations in the different phases of the soil, allowed to conceive the equilibrium isotherms and select the best fit isotherm model. Figure 3.5 presents the obtained results.

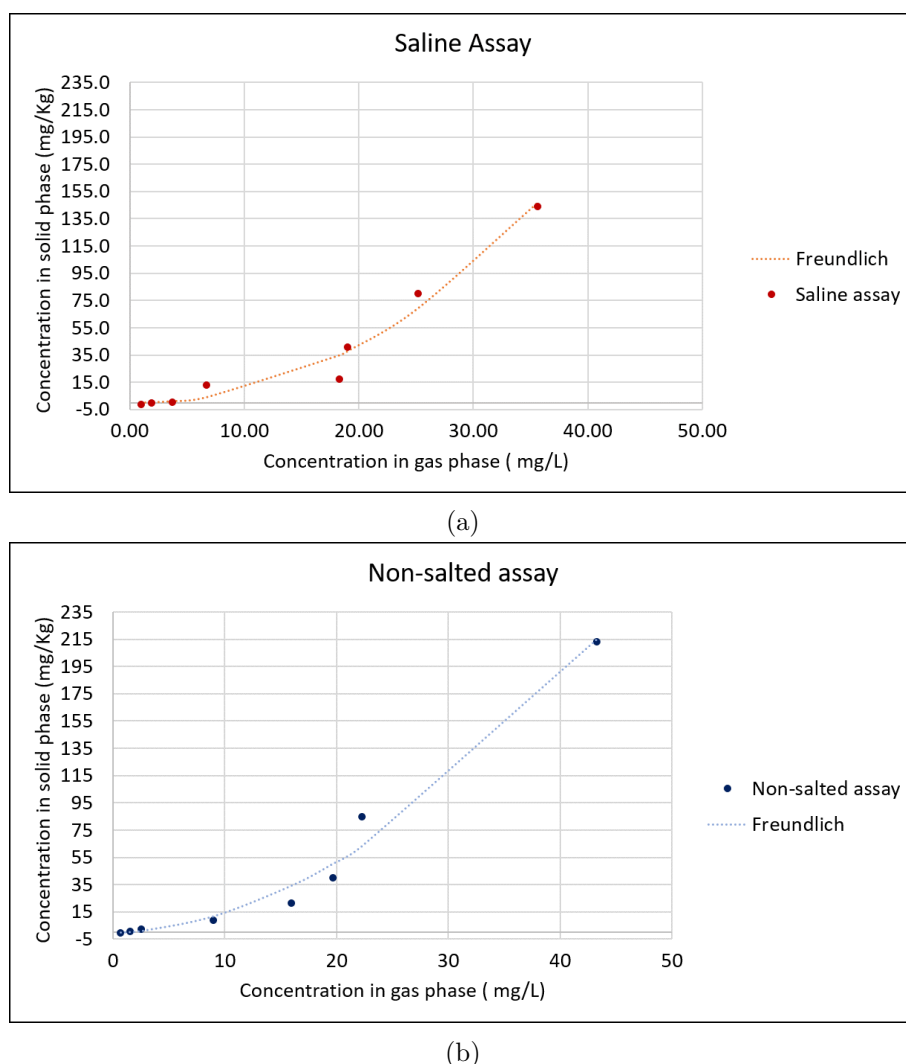


Figure 4.18: Equilibrium isotherms for the residual granitic soil.

Isotherms model adjustment was made by using the program "solver" routine in Excel, which performs a minimization of the sum of squared deviations, variating the parameters K_L and n , for Freundlich model, and K_f and b for Langmuir model.

The model that showed the more adequate adjustment was Freundlich model, for both

assays. As it can be verified in the Table 4.5, Freundlich had better values of R^2 , compared with Langmuir model.

The parameter n presented values higher than the unit, in both cases, evidencing that the adsorption was favorable. Also, K_f parameter was higher for non-salted residual granitic soil, representing a greater capacity for adsorption.

Langmuir model proved to be unsuitable, as the R^2 was considerable lower, compared with Freundlich model.

Table 4.5: Results obtained in the adjustment of the experimental values to the Freundlich and Langmuir models

	Freundlich			Langmuir		
	K_f (l/kg)	n	R^2	K_L (mg/Kg)	b	R^2
Saline assay	0.064	2.164	0.982	0.0002	13539.75	0.888
Non-salted assay	0.213	1.836	0.986	0.0002	18575.6	0.894

5

Conclusions

The soil characterization revealed a slightly acid residual granitic soil, with an extensive particle size distribution and well graded. According to Feret diagram classification, the soil is a loamy sand, with a high content of sand and a very low value of clay. In addition, the soil presented a low content of organic matter (1.88%) and around 51% of saturation water content.

Bioremediation and bioventing tests revealed a consortium with limited degradation capacity, despite having demonstrated in previous studies good degradation efficiencies. However, in the non-salted bioremediation column, a presence of fungi was discovered with a great degradation potential, having eliminated the contaminants in about 20 days. The same was not verified in saline conditions, having observed the death of fungi and the bacteria. According to Qin, in 2012, the high salinity content are related to the inhibition of the metabolic activity, due to the high osmotic potential and changes on the solubility.

The contaminants behavior during the bioventing and bioremediation tests were in concordance with their properties: vapor pressure and Henry's constant, showing higher concentration of benzene in the headspace and more adsorption of ortho-xylene.

Moreover, respirometry detected a slight biological activity. In relation to the first assay, microbial activity was negligible, which was confirmed by the biomass decrease during the assays. Concerning the second assay, more signs of biological activity were verified, in the saline column, compared with the non-salted column, however not related to the contaminants, since there were not evidences of biodegradation. Further, the inoculated saline columns revealed a mark of the salinity by the microorganisms. This mark could not be explained during this research.

Bioventing assays were inconclusive, regarding the implications of the salinity on soil bioremediation.

Bioremediation assay allowed to conclude that the saline environment induce negative effects on the degrading microorganisms.

Concerning the study of contaminants distribution, the increase of the contaminant mass inside the flask, induced an increase of the concentration in the different phases of the soil. However, in the fluid phase was observed a stabilization zone for the middle concentration.

In addition, Freundlich model was, in both cases, considered the most appropriate isotherm model. On the other hand, Langmuir model was not appropriate for the adsorption results.

6

Limitations and Future work

The main limitation to the research was the selected consortium. Despite presenting, in previous studies, very good efficiencies of soil bioremediation, it seems that it lost its capacity to biodegrade high contaminations levels, probably due to their age and conservation conditions. Another limitation was related with the equipment, the gas chromatograph, which presented initially some difficulties in the calibration, which delayed the analysis.

In future work, it is important to collect a fresh consortium and conserve it properly. It is also important, before applying it to column scale remediation tests, to check and optimize their biodegradation capability by performing exploratory bioremediation tests in flasks.

Also, the effect of the salt should be studied for different concentrations, starting with lower concentrations and increasing to above the concentration used, since can occur in soils accumulations of salt concentration, not only from the intrusion of the sea, but from anthropogenic sources. This way, it can be possible to determine the limitations of the microorganisms.

Finally, it would be interesting to explore the biodegradation capability of the fungi discovered during the bioremediation assays.

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Appendix A

Calibration

Appendix 1 refers to the values obtained of the performance of the calibration line

Volume BTEoX (μL)										
	0.4	1	2	4	8	19	28	40	70	
Benzene										
Vol BZ(μL)	0.1	0.25	0.5	1	2	7	10	17.5	25	
BZ (mg L-1)	0.079679	0.199197	0.398394	0.796787	1.593575	5.577512	7.967874	13.9437797	19.91968528	
Peak Areas	38282.3	83758.7	163401.2	317076.1	667648	3148797	5216157	8.57E+06	1.22E+07	
Media	33187.4	86140.9	163347.3	321593.2	612274.3	3198800	5177472	8.79E+06	1.35E+07	
Standard deviation	35734.85	84949.8	163374.3	319334.7	639961.2	3173798	5196815	8676886.35	12859697.95	
RSD (%)	3602.638	1684.47	38.11306	3194.072	39155.12	35357.74	27354.14	154217.6555	912398.3353	
	10.08158	1.9829	0.023329	1.000227	6.118359	1.114051	0.526364	1.777338659	7.095021507	
Toluene										
Vol T(μL)	0.1	0.25	0.5	1	2	7	10	17.5	25	
T (mg L-1)	0.079043	0.197607	0.395214	0.790428	1.580855	5.532994	7.904276	13.83248385	19.76069122	
Peak Area	33332.2	81256.4	144879.4	279491.2	573254.8	2930220	4519721	6894102.6	9419127	
Media	39119.8	87260.8	154410.9	295101.8	538550.3	2781979	4369429	7661334.6	9203983.3	
Standard deviation	33332.2	84258.6	149645.2	287296.5	555902.6	2781979	4444575	7277718.6	9311555.15	
RSD (%)	4092.451	4245.752	6739.788	11038.36	24539.79	104821.9	106272.8	542514.9499	152129.5692	
	12.27777	5.038954	4.503847	3.84215	4.414405	3.767888	2.391069	7.454464507	1.633771875	
Ethylbenzene										
Vol ET(μL)	0.1	0.25	0.5	1	2	7	10	17.5	25	
ET (mg L-1)	0.078679	0.196698	0.393397	0.786793	1.573587	5.507554	7.867935	13.76888622	19.66983746	
Peak Area	25134.9	65246.7	106267.5	214428.1	423713.8	2351041	3594216	4920333.5	6469493.5	
Media	24387.2	71583.2	122330.4	260414.6	425468.1	2153511	3119912	5632089.7	7687476.8	
Standard deviation	24761.05	68414.95	114299	237421.4	424591	2153511	3357064	5276211.6	7078485.15	
RSD (%)	528.7037	4480.582	11358.19	32517.37	1240.477	139674.7	335384.2	503287.6356	861244.2508	
	2.135223	6.549127	9.937261	13.69606	0.292158	6.485905	9.990403	9.538806889	12.16707011	
Ortho-xylene										
Vol oXL(μL)	0.1	0.25	0.5	1	2	7	10	17.5	25	
XL (mg L-1)	0.078134	0.195336	0.390671	0.781342	1.562685	5.469396	7.813423	13.67348978	19.53355683	
Peak Area	22014.4	61665.2	94807.7	197841.4	374705.3	2164431	3317501	4557175.3	5653139.5	
Media	24919.8	67907	108394.1	252597.4	389296.1	1944619	2956589	5007034.3	6215022.9	
Standard deviation	22014.4	64786.1	101600.9	225219.4	382000.7	1944619	3137045	4782104.8	5934081.2	
RSD (%)	2054.428	4413.619	9607.036	38718.34	10317.25	155359.8	255203.1	318098.3495	397311.5624	
	9.332201	6.812602	9.45566	17.19139	2.700847	7.989213	8.135143	6.651848146	6.695418364	

Table A.1: Results obtained of the standard concentration in the gas phase.

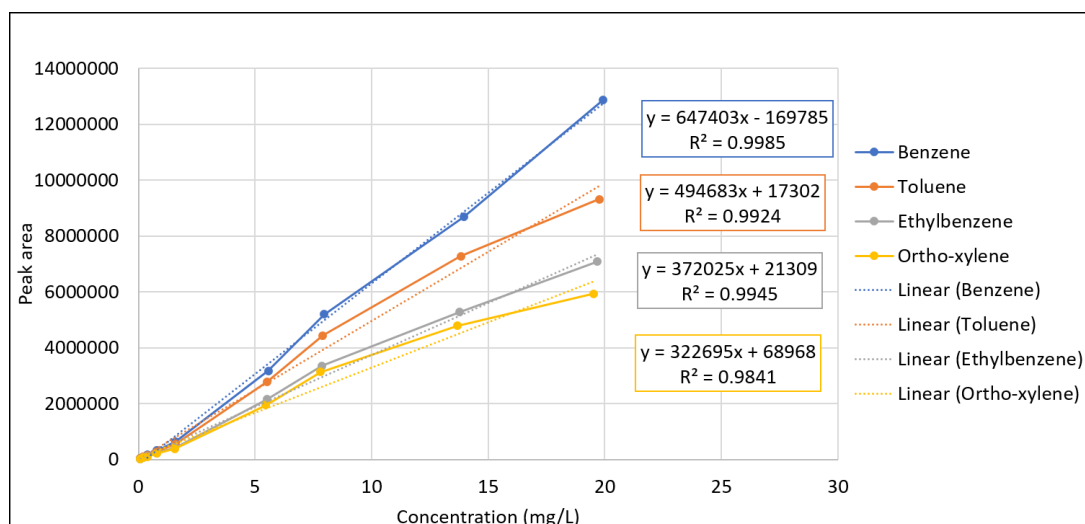


Figure A.1: Calibration line

Appendix B

Remediation tests

Appendix 2 refers to the results obtain of the bioremediation and bioventing results for both assays.

Time (t)	Benzene saline (Bvs1)	Benzene control(BVb)	Toluene saline (Bvs1)	Toluene Control (Bvb)	Ethylbenzene saline (Bvs1)	Ethylbenzene Control (Bvb)	O-Xylene saline (Bvs1)	O-Xylene Control (BVb)
	Cgas	Cgas	Cgas	Cgas	Cgas	Cgas	Cgas	Cgas
0	66.16	17.46	28.01	16.43	11.05	14.13	7.05	9.92
1	15.62	15.50	14.83	14.10	13.66	12.88	10.38	10.52
2	11.32	15.34	9.36	14.49	6.97	11.76	4.91	9.44
3	8.18	9.10	5.69	7.65	4.02	6.04	2.91	4.70
4	9.16	9.14	7.68	8.05	5.99	6.42	4.39	5.21
5	8.49	8.49	6.54	6.54	0.02	6.23	3.36	5.18
6	7.41	7.15	5.94	5.91	4.77	4.66	3.55	3.78
7	6.75	4.96	5.79	4.20	4.89	3.45	3.88	2.91
7	6.18	5.81	4.87	4.72	3.82	3.63	2.90	2.92
9	4.73	5.39	3.90	4.61	3.35	3.87	2.73	3.29
10	4.56	4.35	3.55	3.63	2.62	2.92	2.02	2.45
11	4.38	3.41	3.52	2.76	2.86	2.10	2.27	1.71
12	4.05	3.54	3.30	3.08	2.81	2.67	2.44	2.36
13	2.73	3.66	2.19	2.89	1.67	2.29	1.34	1.90
14	3.06	2.86	2.51	2.33	2.11	1.83	1.69	1.52
15	2.94	2.12	2.38	1.74	1.85	1.37	1.54	1.08
16	2.29	2.36	1.72	1.98	1.56	1.61	1.21	1.57
17	2.42	2.17	1.94	1.75	1.58	1.42	1.17	1.21
18	2.27	1.96	1.87	1.60	1.60	1.28	1.29	1.06
20	1.87	1.59	1.47	1.33	1.18	1.10	0.95	0.89
23	1.75	1.39	1.37	1.22	1.32	1.09	1.02	0.82
25	1.09	1.17	1.02	0.92	0.38	0.61	0.20	0.49

Table B.1: First bioventing assay results

Time (days)	Benzene saline (Bs1)	Benzene control (Bb)	Toluene saline (Bs1)	Toluene Control (Bb)	Ethylbenzene saline (Bs1)	Ethylbenzene Control (Bb)	O-Xylene saline (Bs1)	O-Xylene Control (Bb)
	Cgas	Cgas	Cgas	Cgas	Cgas	Cgas	Cgas	Cgas
0	17.96	0.19	20.30	-0.06	15.76	-0.48	11.45	-0.63
1	17.54	16.69	17.42	16.35	17.90	16.43	14.38	13.83
2	15.20	14.77	13.79	13.04	12.30	11.57	9.65	9.64
3	10.81	12.63	9.24	10.77	7.87	8.77	6.08	7.00
4	15.90	16.11	14.06	13.96	12.03	11.43	9.57	9.54
5	13.83	13.18	11.02	10.44	8.37	7.57	6.36	5.91
6	14.03	12.13	11.62	9.83	9.11	7.14	7.02	5.61
7	14.62	13.45	11.82	9.92	8.75	6.43	6.74	5.03
7	13.35	12.33	10.09	9.01	7.11	5.71	5.30	4.31
9	13.26	12.59	10.34	9.63	7.25	6.46	5.34	5.10
10	13.67	12.58	10.71	9.51	7.72	6.32	5.87	5.01
11	11.47	12.30	7.30	8.98	4.67	5.61	3.60	4.21
12	15.11	12.96	12.66	9.64	9.87	6.44	7.88	5.19
13	10.41	10.78	8.47	7.76	6.32	4.77	4.89	3.74
14	12.01	11.59	9.42	8.11	6.86	5.25	5.19	4.22
15	6.37	13.61	5.80	10.62	4.77	6.93	3.78	5.32
16	10.72	14.50	8.31	11.24	5.87	7.56	4.51	6.07
17	11.50	12.22	8.24	8.39	5.62	5.18	4.28	4.16
18	11.96	11.59	8.64	8.01	5.96	5.00	4.53	3.85
20	10.85	10.16	7.82	6.96	5.55	4.37	4.24	3.51
23	9.85	9.45	6.68	6.52	4.44	4.21	3.21	3.35
25	9.84	8.48	6.57	4.93	4.10	2.29	3.07	1.69

Figure B.1: First bioremediation assay results

tempo (t)	Benzene saline (Bvs2)	Benzene non-salted (BV2)	Toluene saline (Bvs2)	Toluene non-salted (Bv2)	Ethylbenzene saline (Bvs2)	Ethylbenzene non-salted (Bv2)	O-Xylene saline (Bvs2)	O-Xylene non-salted (BV2)
0	Cgas	Cgas	Cgas	Cgas	Cgas	Cgas	Cgas	Cgas
1	5.87	6.88	5.73	6.93	4.14	5.01	3.02	3.66
2	7.39	9.22	9.07	12.17	9.01	13.36	8.30	12.48
3	6.07	7.09	7.06	8.95	6.61	9.31	5.88	8.73
4	9.77	5.82	11.67	7.27	11.35	7.55	10.51	7.17
5	4.73	0.76	5.45	6.42	5.16	6.65	4.69	6.36
6	4.08	4.79	4.76	5.70	4.53	5.68	4.15	5.29
7	3.71	4.24	4.28	5.02	4.19	5.25	3.87	5.05
8	3.07	3.54	3.40	4.21	3.25	4.42	2.93	4.30
9	2.73	3.07	3.06	3.60	3.01	3.81	2.66	3.71
10	2.53	2.79	2.88	3.22	2.88	3.38	2.59	3.32
11	2.50	2.54	2.81	3.00	2.79	3.35	2.48	3.39
12	2.56	2.30	3.10	2.72	3.38	3.04	3.23	3.06
13	2.01	2.03	2.26	2.38	2.34	2.69	2.14	2.66
14	1.99	1.78	2.38	2.06	2.74	2.42	2.65	2.46
15	1.70	1.85	1.82	2.24	1.90	2.62	1.70	2.71
16	1.42	1.47	1.53	1.69	1.61	1.88	1.43	1.83
18	1.35	1.22	1.52	1.28	1.63	1.38	1.43	1.31
19	1.24	1.04	1.43	1.08	1.68	1.20	1.52	1.12
20	1.00	0.91	1.00	0.90	1.07	0.95	0.87	0.86
22	1.09	0.88	1.21	0.87	1.41	0.97	1.20	0.86
23	0.93	0.79	0.96	0.83	1.04	0.92	0.88	0.81
24	0.82	0.76	0.81	0.76	0.92	0.81	0.74	0.71
25	0.76	0.70	0.75	0.70	0.86	0.81	0.72	0.73
26	0.71	0.72	0.69	0.70	0.89	0.77	0.75	0.66
27	0.64	0.64	0.59	0.56	0.58	0.70	0.56	0.46
28	0.68	0.62	0.72	0.51	0.95	0.58	0.84	0.50
30	0.57	0.59	0.50	0.51	0.65	0.55	0.50	0.43
31	0.48	0.49	0.48	0.45	0.50	0.49	0.46	0.43
32	0.49	0.47	0.38	0.40	0.49	0.43	0.37	0.31
33	0.45	0.48	0.29	0.33	0.36	0.36	0.23	0.22
33	0.44	0.44	0.30	0.30	0.37	0.33	0.25	0.17

Figure B.2: Second bioventing assay results

Time (days)	Benzene non-salted (B2)	Benzene saline(Bs2)	Benzene non-salted control (Bb2)	Toluene non-salted (B2)	Toluene saline(Bs2)	Toluene non-salted control (Bb2)	Ethylbenzen e non-salted (B2)	Ethylbenzene saline(Bs2)	Ethylbenzene non-salted control (Bb2)	O-xylene non-salted (B2)	O-xylene saline(Bs2)	O-xylene non-salted control (Bb2)
	Cgas	Cgas	Cgas	Cgas	Cgas	Cgas	Cgas	Cgas	Cgas	Cgas	Cgas	Cgas
0	7.25	7.29	6.82	11.93	8.72	6.97	8.82	12.17	6.16	7.11	14.54	5.14
1	3.88	9.82	9.54	11.81	13.21	12.55	22.38	15.54	14.57	27.16	15.20	13.61
2	1.30	9.97	9.31	8.34	13.22	11.80	11.61	14.45	12.59	11.63	14.20	12.15
3	0.45	9.29	8.98	6.26	11.88	11.01	10.68	12.60	11.24	10.73	12.16	10.47
4	0.34	8.56	8.94	5.03	10.66	10.65	9.48	10.64	10.45	9.58	9.89	9.71
5	0.32	8.74	8.51	4.32	10.87	9.82	8.39	10.76	9.01	8.40	10.32	8.17
6	0.29	7.62	8.79	3.69	9.28	9.60	7.77	9.40	8.64	7.85	9.00	7.59
7	0.29	6.98	9.51	3.21	8.35	10.96	6.74	7.94	10.11	6.89	7.40	9.17
8	0.29	6.94	6.95	2.80	8.24	8.26	5.82	7.88	7.88	5.95	7.45	7.48
9	0.31	7.69	9.19	2.73	9.67	10.29	5.85	9.88	9.19	6.40	9.59	8.31
10	0.29	6.62	7.83	2.68	7.95	8.25	5.74	7.63	6.83	6.75	7.16	5.99
11	0.29	6.24	8.17	2.24	7.25	8.61	4.74	6.73	7.07	6.03	6.31	6.20
12	0.28	5.54	7.36	1.76	6.26	7.59	3.70	5.84	6.49	5.24	5.69	5.97
13	0.29	6.09	7.26	1.30	7.16	7.06	2.92	6.89	5.39	4.89	6.47	4.51
14	0.28	5.60	7.16	0.51	6.27	7.15	1.95	5.73	5.84	4.71	5.47	5.28
15	0.27	5.29	7.31	0.18	5.84	7.43	1.11	5.29	6.31	2.37	5.07	5.85
16	0.27	5.17	6.67	0.08	5.70	6.29	0.44	5.10	4.65	0.53	4.69	4.02
18	0.26	4.53	6.03	0.04	4.70	5.45	0.33	3.95	4.17	0.45	3.43	3.59
19	0.28	4.30	4.30	0.07	4.34	4.34	0.31	3.58	3.58	0.35	3.09	3.09
20		4.13	5.82		4.22	5.03		3.60	3.76		3.16	3.19
22		4.15	6.00		4.25	5.53		3.40	4.22		3.06	3.83
23		3.85	5.78		3.82	5.12		3.21	4.01		2.86	3.51
24		3.72	5.77		3.57	4.98		3.02	3.88		2.66	3.37
25		3.73	6.10		3.61	5.34		2.99	4.14		2.63	3.59
26		3.60	5.71		3.48	4.81		2.85	3.51		2.54	3.03
27		3.52	5.22		3.35	4.08		2.74	2.65		2.43	2.11
28		3.41	5.12		3.18	4.08		2.61	2.95		2.27	2.52
30		3.21	4.99		2.88	3.88		2.23	2.73		1.94	2.33
31		3.23	5.02		2.89	3.90		2.19	2.68		1.89	2.32
32		2.97	4.68		2.53	3.54		1.89	2.49		1.53	2.09
33		2.98	4.59		2.59	3.43		2.01	2.36		1.69	1.99
35		2.76	4.35		2.20	3.12		1.59	2.12		1.18	1.71
38		2.63	4.51		2.12	3.40		1.58	2.36		1.27	2.02
41		2.51	4.30		2.05	3.37		1.53	2.32		1.30	2.02

Figure B.3: Second bioremediation assay results

Appendix C

Isotherms

Appendix 3 presents the results obtain in the adsorption assays.

Saline Assay						
Vgas=315 cm ³		Vsoil=604.9cm ³		Vwater=175cm ³		H'=0.289 ρ=0.868 Msoil=525g
M _{BTEx} (mg)	Cgas (mg/l)	Mgas(mg)	Caq(mg/l)	Maq(mg)	Ms(mg)	Cs(mg/l)
0.35	0.97	0.30	3.34	0.58	-0.54	-1.03
1.74	1.93	0.61	6.66	1.17	-0.04	-0.08
3.47	3.70	1.16	12.79	2.24	0.07	0.13
13.02	6.69	2.11	23.15	4.05	6.86	13.07
26.04	18.31	5.77	63.35	11.09	9.18	17.49
39.05	19.04	6.00	65.88	11.53	21.53	41.01
65.09	25.15	7.92	87.02	15.23	41.94	79.89
108.48	35.61	11.22	123.23	21.57	75.70	144.19

Figure C.1: Adsorption results of saline assay

Non-salted assay						
Vgas=315 cm ³		Vsoil=604.9cm ³		Vwater=175cm ³		H'=0.289 ρ=0.868 Msoil=525g
M _{BTEx} (mg)	Cgas (mg/l)	Mgas(mg)	Caq(mg/l)	Maq(mg)	Ms(mg)	Cs(mg/l)
0.35	0.68	0.21	2.36	0.41	-0.28	-0.53
1.74	1.53	0.48	5.31	0.93	0.32	0.62
3.47	2.51	0.79	8.68	1.52	1.16	2.22
13.02	8.94	2.82	30.93	5.41	4.79	9.12
26.04	15.96	5.03	55.22	9.66	11.35	21.61
39.05	19.65	6.19	68.00	11.90	20.96	39.93
65.09	22.25	7.01	76.99	13.47	44.60	84.96
217.50	43.28	13.63	149.75	26.21	112.03	213.40

Figure C.2: Adsorption results of non-salted assay