LIFE BIOREST

Environmental guideline for contaminated soil bioremediation

THE STRATEGY TO RECLAIM GREEN LANDS FOR THE COMMUNITY

Consorzio Italbiotec

Environmental Guideline for Contaminated Soil Bioremediation

The strategy to reclaim green lands for the community

Release Notes

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Glossary

Aerobic	An environment that has a partial pressure of oxygen similar to normal atmospheric conditions.
Anaerobic	An environment without oxygen.
Biodegradation	The breakdown of organic substances by microorganisms.
Bioemulsifier	Molecules able to efficiently emulsify two immiscible liquids such as hydrocarbons or other hydrophobic substrates even at low concentrations but that, in contrast, are less effective at reducing tension.
Biopile	A pile of contaminated soils used to reduce concentrations of pollutants e.g. petroleum constituents in excavated soils through the use of biodegradation.
Bioreactor	It refers to any manufactured device or system that supports a biologically active environment. A bioreactor is a vessel in which a chemical process is carried out, involving microorganisms or biochemically active substances derived from such organisms. This process can either be aerobic or anaerobic.
Bioremediation	The process used to treat contaminated matrices, including water, soil and subsurface material, by altering environmental conditions to stimulate the growth of microorganisms that degrade or transform the target organic contaminants
Bioslurping	Combination of elements of bioventing and vacuum-enhanced pumping of free product to recover free product from the groundwater and soil, and to bioremediate soils.
Biosparging	In-situ remediation technology which uses indigenous microorganisms to biodegrade organic constituents in the saturated zone. Air (or oxygen) and nutrients (if needed) are injected into the saturated zone to increase the biological activity of the indigenous microorganisms.
Biostimulation	A process involving the modification of the environment to stimulate existing bacteria capable of bioremediation. For example, the addition of nutrients, oxygen, or other electron donors and acceptors.
Bioventing	The process of stimulating the natural in situ biodegradation of contaminants in soil by supplying air or oxygen to existing soil microorganisms. In this process, low airflow rates are used, stimulating biodegradation and minimising volatilisation.
Ex situ	Out of the original position (i.e. excavated).
Fungi	A group of diverse and widespread unicellular and multicellular eukaryotic organisms. Many species are well-known for their capability to degrade different types of aromatic and aliphatic pollutants.
In situ	In place, without excavation
Landfarming	Ex-situ waste treatment process that is performed in the upper soil zone or in biotreatment cells. Contaminated soils, sediments, or sludges are transported to the land farming site, incorporated into the soil surface and periodically turned over (tilled) to aerate the mixture.
Mycoremediation	Process of using fungi to degrade or sequester contaminants in the environment. Through the stimulation of the microbial and enzyme activity, mycelium reduces toxins in-situ.
Phytoremediation	Treatment of pollutants or waste (e.g. in contaminated soil or groundwater) using green plants that remove, degrade, or stabilise the undesirable substances, such as toxic metals and other contaminants.
Surfactant	Surfactants may be added to alter the properties of solution interfaces, enabling the access of hydrocarbons to the microorganisms. This is referred to as "Surfactant Aided Bioremediation" or "Surfactant Enhanced Bioremediation" (SEB).
Windrow	Specific ex situ remediation technique based on the periodic turning of piled polluted soil to increase bioremediation by enhancing the degradation activity of microorganisms.

Acronyms

BTEX	Benzene, Toluene, Ethylbenzene and Xylene
CHC	Chlorinated Hydrocarbons
CSIC	Consejo Superior de Investigaciones Científicas
ELD	Environmental Liability Directive
FAO	Food and Agriculture Organization
ICPE	Installation Classée pour la protection de l'environnement
IPCC	Integrated Pollution and Prevention Control Directive
ITPS	Intergovernmental Technical Panel on Soils
MEDDE	Ministry of Ecology, Sustainable Development and Energy
ORC	Oxygen Releasing Compound
PAH	Polycyclic Aromatic Hydrocarbon
PCB	Polychlorinated Biphenyls
PCDD	Polychlorinated Dibenzo-p-dioxins
PCDF	Polychlorinated Dibenzofurans
PCE	Perchloroethylene, tetrachloroethylene or tetrachloroethene
PFOS	Perfluorooctane Sulfonate
POP	Persistent Organic Pollutant
SIN	Site of National Interest
STS	Soil Thematic Strategy
SVOC	Semi-Volatile Organic Compound
TCE	Trichloroethylene, trichloroethene
VOC	Volatile Organic Compound

Executive summary

The adoption of the 17 Sustainable Development Goals (SDGs) by the 193 UN member states in 2015, contributed to the definition of integrated solutions to face the main global challenges to protect the planet and ensure a sustainable future. Soil degradation represents one of the main challenges recognised both at European and global level, and many of the SDGs refer to land and soil preservation and protection. Since soil is considered a non-renewable resource, maintaining its health is essential to promote the basic functions of supplying essential nutrients, water, oxygen and support for plants. Despite the vital importance of soil, its improper use and management, mainly due to anthropogenic activities, has led to high levels of pollution which can cause serious consequences. Unsustainable soil management drove the European Commission in 2006 to adopt the Soil Thematic Strategy[1] (22 September 2006) to give protection to all soils across the EU. In 2014, the Commission decided to withdraw the proposal for a Soil Framework Directive but, with the adoption of the Seventh Environment Action Programme (7th EAP) of January 2014, soil degradation was recognised as a severe issue.

As reported by the Joint Research Centre of the European Commission, 650,000 contaminated sites have been registered in the inventories of the 28 Member States, where reclamation treatments have been carried out or are ongoing. Currently, 65,500 sites have been subjected to corrective measures. The main contaminants present in soil are heavy metals, mineral oils, volatile organic compounds and polycyclic aromatic hydrocarbons. Despite these high-level initiatives, policies targeted at preventing land and soil degradation remain fragmented, relying on sectoral policies.

This Guideline has the objective of presenting to the local authorities the collected and elaborated recommendations and results obtained through the LIFE BIOREST project.

The LIFE BIOREST project was conceived to demonstrate the efficiency and cost-effectiveness of an innovative and sustainable solution for the bioremediation of hydrocarbon-contaminated soils, based on the use of bacterial and fungal strains with high degrading potential, through the valorisation of agri-food industry by-products. The final goal of the project is to restore the ecological functions of soils, prevent the loss of fertility, biodiversity and resilience and reclaim new green areas back for the community.

This document has been developed to provide a model for the application of a bioremediation technique in those municipalities which are affected by contaminated sites, using the case-study of the Fidenza site, where the LIFE BIOREST project took place.

The partnership can count on the experience of Consorzio Italbiotec, acting as project coordinator, Actygea Srl, Agenzia regionale per la Prevenzione, l'Ambiente e l'Energia - ARPAE, University of Turin, Università Cattolica del Sacro Cuore, Agencia Estatal Consejo Superior de Investigaciones Científicas (Spain) and SATT- SAYENS (France).

During the LIFE BIOREST project, dissemination activities at European level played a very important role, to supporting the Soil Thematic Strategy and showing the advantages of a biological approach to legislators, public authorities, industries and the community.

For this reason, LIFE BIOREST proposes a model for the application of the bioremediation treatment to the contaminated sites, with the potential for it to be diffused around Europe.

[1] Soil Thematic Strategy (COM(2012) 46) - 2006: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:52012DC0046

1. Introduction

As reported by FAO and ITPS, soil contamination is the condition in which the presence of a chemical or substance that is out of place and/or present at a higher than normal concentration, with adverse effects on any non-targeted organism, can be observed.[2] As it cannot often be visually perceived or directly assessed, soil contamination is considered a "hidden danger", causing serious consequences.

It impairs plant metabolism, thus impacting on food security and reducing crop yields, as well as by making crops unsafe for consumption. The primary sources of soil contamination are anthropogenic, resulting in the accumulation of contaminants in soils that may reach levels of concern (Cachada, Rocha-Santos and Duarte, 2018). Soil contaminated with dangerous elements, such as heavy metals and organic chemicals like PCBs (polychlorinated biphenyls), PAHs (polycyclic aromatic hydrocarbons) or pharmaceuticals, contribute to causing serious risks to human health. The diversity of contaminants is subject to constant evolution due to agrochemical and industrial developments and the transformation of organic compounds in soils into secondary metabolites through biological activity makes identifying the contaminants both difficult and expensive. Moreover, the physical properties of soil as structure, texture, particle arrangement, porosity, etc. also affect mobility, bioavailability, and the time that contaminants are present in the soil.

As reported by the Joint Research Centre of the European Commission (Van Liedekerke et al., 2014), about 39% and 29% of contaminants in European soils are heavy metals and mineral oils, respectively (**Fig. 1**), as a result of petroleum transport, storage and refining or accidents (Gallego et al., 2001). Benzene, toluene, ethylbenzene, and xylene (BTEX) make up the category of volatile organic compounds (VOCs), found in petroleum-derived products such as gasoline and represent 11% of soil contaminants. BTEX substances have high mobility because they are soluble in water and volatile. Chlorinated hydrocarbons (CHCs) are used mainly for the manufacturing of synthetic solvents and insecticides. As they are able to bioaccumulate, they can also be found in human tissues. They account for 9% of soil contaminants. The most common source of cyanide contamination is former gas work sites. However, cyanide contamination is also associated with electroplating factories, road salt storage facilities, and gold mine tailings (Kjeldsen, 1999) and, together with phenolic pollutants related to the oil-shale industry, account for 2% of soil contaminants. Polycyclic aromatic hydrocarbons (PAHs), which represent 12% of soil contaminants, are ubiquitous environmental pollutants generated primarily during the incomplete combustion of organic materials (e.g. coal, oil, petrol, and wood). PAHs accumulate in soils because of their persistence and hydrophobicity; they tend to be retained into the soil for long periods. For this reason, most PAHs are components of persistent organic pollutants (POPs) and are widespread in air, water, soils, and sediments (Lin et al., 2013). Although PAH emissions from anthropogenic activities predominate, some PAHs in the environment originate from natural sources such as open burning, natural losses or seepage of petroleum or coal deposits, and volcanic activities. Primary anthropogenic sources of PAHs include residential heating, coal gasification and liquefying plants, carbon black, asphalt production, related activities in petroleum industry as well as motor vehicle exhausts. Traffic emissions and fossil fuel combustion are the main identified sources of PAHs in urban areas (Fabiańska et al., 2016; Keyte et al., 2013).

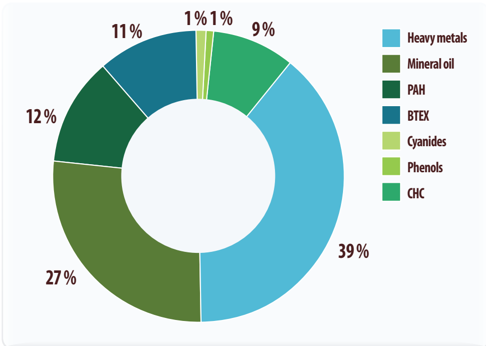

Figure 1 Overview of contaminants affecting European soils

[2] Status of the World's Soil Resources (SWSR) - Main Report. FAO & ITPS, 2015. http://www.fao.org/3/a-i5199e.pdf

1.1 Scope of this Guideline

Industrialised economies and developing countries are affected by soil contamination originating from extractive and industrial activities, improper waste disposal, and mechanised agriculture that could have impacts on crop productivity and human health. Since soil contamination is an important and, sometimes, underestimated issue, this Guideline aims to present to the local authorities the collected and developed recommendations and results obtained through the LIFE BIOREST project. The project was conceived to demonstrate the efficiency and cost-effectiveness of an innovative and sustainable solution for the bioremediation of hydrocarbon-contaminated land, based on the use of bacterial and fungal strains with high degrading potential, through the valorisation of agri-food industry by-products.

The information in this document is not intended to provide detailed guidance on the design of a bioremediation treatment program, but it is aimed to provide a model for the application of a bioremediation technique in those municipalities which are affected by contaminated sites, using the case-study of the Fidenza site, where the LIFE BIOREST project took place.

Furthermore, the Guideline aims to support public-private partnership and to show the advantages and drawbacks of the implanted technique that can be presented to industries, public bodies, national governments and the community.

The European legislative framework addressing soil contamination has been analysed with the aim of comparing the legislative adaptation of the three countries, where LIFE BIOREST has been implemented (Italy, Spain and France), to the European directives.

As analysed in-depth in the document, in the 3 EU nations, hydrocarbon contamination is the most common emergency in terms of contamination and the need for remediation.

These contaminants, as compared to trace elements, can be degraded by microorganisms and plants. For this reason, LIFE BIOREST could represent a model for the application of the bioremediation treatment to the contaminated sites, with the potential to be diffused around Europe.

1.2 European legislative framework on soil contamination

Legal requirements for the general protection of soil have not been agreed at the European Union (EU) level and, currently, only a few EU Member States possess specific legislation on soil protection.

Therefore, soil is not subject to a complete and consistent set of rules within the EU. Existing EU policies in areas such as agriculture, water, waste, chemicals, and prevention of industrial pollution indirectly contribute to the protection of soils, but they are not enough to guarantee an appropriate level of protection for all soils present in Europe. Since soil is continuously used in an unsustainable way, the European Commission in 2006 adopted the so-called Soil Thematic Strategy[3] (22 September 2006) with the aim of giving protection to all soils across the EU. In 2014, the Commission decided to withdraw the proposal for a Soil Framework Directive but, with the adoption of the Seventh Environment Action Programme[4] of January 2014, soil degradation was recognised as a serious issue. It indicates that by 2020, land should be managed in a sustainable way in all the EU Member States, the soil should be adequately protected, and the remediation of contaminated sites should be well-developed. Different European Directives provide indirect controls on soil contamination and advice for its management, such as the Integrated Pollution and Prevention Control Directive (IPPC 2008/1/ EC), the Waste Framework Directive (2008/98/EC) and Landfill Directive (99/31/EC)). Furthermore, the Directive on Industrial Emissions (IED 2010/75/ EU), which abrogated the IPCC Directive in 2014, provides a regulatory framework to prevent emissions to the soil from large industrial plants reaching the soil.

The Table below represents an overview of existing national targets. These include a variety of forms, such as referencing timelines for remediation of historic contamination or specific management steps or lists of national priority sites. In total, 17 countries report official policy targets for the management of Contaminated Sites.

[3] Soil Thematic Strategy (COM(2012) 46) - 2006: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:52012DC0046
[4] 7th EAP – 2013: https://ec.europa.eu/environment/action-programme/

Table 1. Overview of national policies and EU directives specifically addressing specifically soil contamination (Source: Status of local soil contamination in Europe - JRC Technical Report, 2018).

1986	Sewage-sludge directive[5] Regulation of the use of sewage sludge in agriculture in order to prevent harmful effects on soil and establishing limit values of heavy metals in soils.
1987	**Netherlands** - Soil protection Act
1988	
1989	**Austria** - Law on the remediation of contaminated sites
1990	
1991	Nitrates directive[6] Protection of surface water and groundwater against contamination by nitrates from agricultural sources.
1992	Habitats directive[7] Achievement of a favourable conservation status throughout the natural range within the EU, and to reduce the pollution of habitats, thus reducing soil contamination.
1993	
1994	**Finland** - Waste Act
1995	**Estonia** - Contaminated site management **Belgium (Flanders)** - Decree on soil remediation and soil protection **Switzerland** - Environmental Protection Act (EPA)
1996	**Hungary** - Decision No. 2205/1996 (VII.24) adopted the national environmental remediation programme (before being part of EU) **Slovenia** - Decree on limit values, alert thresholds and critical levels of dangerous substances into the soil
1997	
1998	**Germany** - Federal soil protection Act
1999	Landfill directive[8] Prevention/reduction of the negative effects of waste landfills on the environment during the whole life cycle of the landfill. **Denmark** - Act on soil contamination **Italy** - Regulation laying down criteria, procedures and methods for the safety, reclamation and restoration of polluted sites **Luxembourg** - Law on classified establishments
2000	Water framework directive[9] Prevention and reduction of pollution; main pollutants are listed, and thresholds established. **France** - Environmental Code **United Kingdom** - Contaminated land Regime (Part 2A of environmental protection act, 1990) **Finland** - Environmental Protection Act

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[5] Council Directive 86/278/EEC on the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:31986L0278
[6] Council Directive 91/676/EEC concerning the protection of waters against pollution caused by nitrates from agricultural sources: https://eur-lex.europa.eu/legal-content/EN/TXT/?qid=1561542776070&uri=CELEX:01991L0676-20081211
[7] Council Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:31992L0043
[8] Council Directive 1999/31/EC on the landfill of waste: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:31999L0031
[9] Directive 2000/60/EC establishing a framework for Community action in the field of water policy: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32000L0060

2001	Strategic environmental assessment directive[10] Reduction of the environmental impacts from plans and programmes in the environment, including soils. **Latvia** - Law on pollution
2002	**Cyprus** - Water and soil contamination control Law
2003	
2004	Environmental Liability Directive (ELD)[11] Establishment of a framework based on the polluter-pays principle (PPP) to prevent and remedy environmental damage to soil, ecosystems and water resources, if human health is affected. **Belgium (Brussels-Capital)** - Ordinance on the management and clean-up of soils **Belgium (Wallonia)** - Decree on the management of soils **Slovakia** - Soil protection Act **Sweden** - Regulation on compensation for contamination damage and state aid for remedial (implementing Swedish environmental code of 1999)
2005	**Hungary** - Decree on rules concerning the screening surveys of remedial site investigation **Spain** - Decree on defining soil polluting activities and criteria
2006	Thematic strategy for soil protection (STS)[12] Protection of soils by preventing soil degradation and restoring degraded soils, included those contaminated. Waste-management extractive industries directive[13] Introduction of measures to prevent or minimise any adverse effects on the environment and health arising from the management of waste from extractive industries. **Ireland** - Energy Act. Historic mine sites - inventory and risk classification **Italy** - Environmental Code **Lithuania** - Regulations on the treatment procedures for contaminated sites
2007	**Bulgaria** - Soil Act **Finland** - Government Decree on the assessment of soil contamination and remediation needs **Romania** - Decree on remediation **Slovakia** - Act on the prevention and remedying of environmental damage
2008	Waste framework directive[14] Provision of the basis of remediation of historical contaminated waste-disposal sites. **Czech Republic** - Act concerning the prevention of environmental harm and its rectification
2009	Pesticide framework directive[15] Prevention of contamination of the environment by pesticides. **Belgium (Brussels-Capital)** - Decree on soil remediation and soil management of 5 March 2009 amended 23 June 2017
2010	Industrial emissions directive[16] Prevention, reduction and elimination (when possible) of the pollution arising from industrial activities. Member States are committed to establish inventories of sulphur dioxide (SO_2), nitrogen oxides (NOx) and dust emissions and produce a baseline report to establish the state of soil and groundwater contamination. **Serbia** - Regulation on the programme for systematic monitoring of the soil quality, indicators for evaluation of soil degradation and methodology for preparation of remediation program

[10] Directive 2001/42/EC on the assessment of the effects of certain plans and programmes on the environment: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32001L0042
[11] Directive 2004/35/CE on environmental liability with regard to the prevention and remedying of environmental damage: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=LEGISSUM%3Al28120
[12] Thematic Strategy for Soil Protection [SEC(2006)620] [SEC(2006)1165]: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A52006DC0231
[13] https://ec.europa.eu/environment/waste/mining/index.htm
[14] Directive 2008/98/EC on waste and repealing certain Directives: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32008L0098
[15] Directive 2009/128/EC establishing a framework for Community action to achieve the sustainable use of pesticides: http://www.europarl.europa.eu/RegData/etudes/STUD/2018/627113/EPRS_STU(2018)627113_EN.pdf
[16] Directive 2010/75/EU on industrial emissions (integrated pollution prevention and control): https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32010L0075

2011	Environmental impact assessment directive[17] Assessment of the environmental effects of public and private projects that are likely to have significant effects on the environment. Biodiversity Strategy[18] Reduction of the loss of biodiversity and ecosystem services in the EU as well as contribution to stop global biodiversity declining by 2020, by promoting healthy soils. **Spain** - Law on waste and contaminated soils
2012	**Malta** - National Environment Policy
2013	
2014	**Croatia** - Ordinance on the protection of agricultural land against pollution
2015	**Serbia** - Law on soil protection
2016	Mercury regulation[19] Identification and evaluation of sites contaminated with mercury, including an inventory of contaminated sites and inclusion of a list of the main mercury compounds.
2017	**Greece** - Law for the protection and sustainable use of soil (under preparation) **Poland** - Assessment of land surface contamination (under preparation) **Portugal** - Contamination prevention and soil remediation legal programme (under preparation) **Slovenia** - Decree on status of soil and rules on status of soil (under preparation)

1.3 Environmental law for soil contamination in Italy

In Italy, soil contamination represents a widespread problem due to industrial areas, landfills, commercial and extractive areas. Despite the seriousness of incidence and health damages shown by the scientific literature (Brevik, 2013; Burgess, 2013; Jordão et al., 2006), the issue of soil contamination does not seem to be perceived by public opinion in the same way as air and water pollution, even if indirectly linked to these. The reduced perception of an environmental emergency in public opinion is confirmed by the FAO report (Rodríguez-Eugenio et al. 2018), which defines soil pollution as a "hidden reality", being more difficult to identify, measure and study over the years.

The evolution of legal provisions, in the matter of contamination reduction and treatment, suffers from this different perception. The first step against environmental contamination is included in Law No. 615/1966[20], regarding "Measures against atmospheric pollution", aimed to regulate and reduce the emissions of fumes, gas, dust and smells from industries and means of transport, recognising the indirect damage to human's health.

With the introduction of the Law No. 319/1976[21] (then abrogated by the Legislative Decree No. 152/2006[22]), regulations for water protection from pollution were defined, representing a starting point for an increasing awareness of environmental issues which has brought about the creation of a specific Ministry of environmental politics. The Ministry for Environment, Land Protection and Sea, established in 1986 with the measure No. 349[23], is charged with ensuring that the promotion, conservation and recovery of environmental conditions comply with the fundamental interests of collectivity and with quality of life, as well as the conservation and valorisation of the national natural heritage and the defense of natural resources from pollution (Art.1).

The establishment of this governing body coincides with the definition of a policy framework for constant territorial monitoring in the matter of environmental pollution.

[17] Directive 2004/35/CE on environmental liability with regard to the prevention and remedying of environmental damage: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32004L0035
[18] Communication from the Commission {COM/2011/244 final} Our life insurance, our natural capital: an EU biodiversity strategy to 2020 https://ec.europa.eu/environment/nature/info/pubs/docs/brochures/2020%20Biod%20brochure%20final%20lowres.pdf
[19] Regulation (EU) 2017/852 on mercury, and repealing Regulation (EC) No 1102/2008: https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32017R0852
[20] https://www.gazzettaufficiale.it/atto/serie_generale/caricaDettaglioAtto/originario?atto.dataPubblicazioneGazzetta=1966-08-13&atto.codiceRedazionale=066U0615&elenco30giorni=false
[21] http://www.reteambiente.it/repository/normativa/761_legge_merli.pdf
[22] https://www.gazzettaufficiale.it/atto/serie_generale/caricaDettaglioAtto/originario?atto.dataPubblicazioneGazzetta=2006-04-14&atto.codiceRedazionale=006G0171
[23] https://www.minambiente.it/sites/default/files/legge_08_07_1986_349.pdf

With Law No. 22/1997[24] (abrogated by the Legislative Decree 152/2006 too), EU Directives regarding waste have been tansposed, introducing the concept of waste as a resource.

The first law regarding the environmental remediation of soil is Ministerial Decree No. 471/1999[25], which defines the criteria and procedures for identification, securing, remediation and environmental restoration of polluted sites.

Legislative Decree n. 152/2006[26], in the matter of Environmental Legislation, replaced the previous Decree No. 471/1999. The new regulations redefine the administrative procedures which have the primary aim of promoting a high level of quality of life, to be realised by the protection and improvement of environmental conditions and rational use of the natural resources (Art. 2).

In the Legislative Decree 152/2006, a contaminated site is defined as a site in which the values of risk threshold concentrations, determined through the application a risk analysis procedure, are exceeded.

This definition refers to all the areas in which, because of ongoing or concluded human activities, an altered level of soil, subsoil and groundwater characteristics has been established, thus representing a risk for human health. The Legislative Decree 152/2006 specifies the typology of contaminated soils present on the territory and the administrative procedures regarding their identification and management.

1.4 Environmental law for soil contamination in Spain

In Spain, the main regulations governing soil contamination are included in the law No. 22/2011[27] on Waste and Contaminated Soils, and the Spanish Soil Decree No. 9/2005[28] on the Creation of a List of Potentially Land-Pollutant Activities and the Criteria for Declaring Contaminated Soils.

The Spanish Soil Decree presents a regulatory framework for the definition of potentially contaminating industrial activities and indicates the methodology for the determination of generic benchmarks of contaminants, mainly derived by risk analysis application. The Decree includes a list of benchmarks for 60 priority substances. It considers ecological and geological diversities of soils in the different Spanish Regions, defying a flexible approach that comprises further in-depth levels.

In the Decree, 3 different typologies of soil uses are taken into consideration: industrial, residential and natural, considering human targets and, for the third typology, also ecosystem targets.

Law 22/2011 on waste and contaminated soils contains a requirement for the establishment of a register of remediated sites. Regional authorities are responsible for it. Other rules deal with soil pollution from different perspectives, such as Royal Legislative Decree No. 1/2016[29], on Integrated Pollution Prevention and Control and the Law No. 26/2007[30] on Environmental Liability.

The declaration of land as polluted, based on the concept of risk (for human health or the environment) and land uses, shall be made by regional authorities on the basis of the criteria set forth in Decree No. 9/2005, which distinguishes between industrial, urban or other uses of the land.

The persons obliged to clean up the site are the polluters, the owner of the polluted site and eventually the possessor.

The declaration of soil as contaminated must be included within the Property Registry and can only be removed when the regional authorities confirm that the clean-up has been duly carried out and the risk to human health or the environment is absent.

[24] http://www.bosettiegatti.eu/info/norme/statali/1997_0022.htm
[25] https://www.gazzettaufficiale.it/eli/id/1999/12/15/099G0540/sg
[26] https://www.gazzettaufficiale.it/atto/serie_generale/caricaDettaglioAtto/originario?atto.dataPubblicazioneGazzetta=2006-04-14&atto.codiceRedazionale=006G0171
[27] Waste and Contaminated Soils
https://www.global-regulation.com/translation/spain/1436517/law-22-2011%252c-28-july%252c-waste-and-contaminated-soils.html
[28] https://www.global-regulation.com/translation/spain/1448611/royal-decree-9-2005%252c-of-14-january%252c-which-establishes-the-relationship-of-potentially-polluting-activities-of-the-soil-and-the-criteria-and-standa.html
[29] https://gettingthedealthrough.com/area/13/jurisdiction/21/environment-spain/#targetText=There%20is%20a%20system%20of,industries%20that%20meet%20certain%20parameters.
[30] https://iclg.com/practice-areas/environment-and-climate-change-laws-and-regulations/spain

1.5 Environmental law for soil contamination in France

The only law for soil contamination management and remediation in France is Law No. 19/1976[31], regarding Classified Installations for the Protection of the Environment (the ICPE law) which has been assimilated into the Environment Act of September 21, 2000[32] and by Directive 2004/35/EC[33] on environmental liability regarding the prevention and remediation of environmental damage (Environmental Liability Directive). The latter has been implemented in France by the Law relating to Environmental Liability and adaptation of various provisions of the EU Environmental Law of 2008[34], which relates to environmental damage caused to the soil that presents a risk to human health.

In the matter of ICPE law, a national strategy including the inventory of contaminated sites and guidelines for their characterisation has been introduced. This law defines the productive activities, such as industries, laboratories, yards, which could have drawbacks for public health, agriculture, environment and landscape protection.

National policy and the measures to be applied are defined by two key documents represented by the circulars from the Ministry of the Environment of December 1993 and of December 1999[35], defining the main features of a national policy for contaminated sites. In particular, the circular of 1999 indicates the principles for the identification of remediation objectives, based on detailed risk analysis and technical-economic evaluation of the alternatives of intervention.

The Ministry of Ecology, Sustainable Development and Energy (MEDDE) is responsible for defining public policy on the subject of contaminated land, whether the contamination is natural or human-generated and whether it relates to ICPE policy or not.

[31] https://aida.ineris.fr/consultation_document/2193
[32] http://www.eugris.info/FurtherDescription.asp?e=183&Ca=1&Cy=3&DocID=B&DocTitle=Policy_and_regulatory&T=France#targetText=France%20has%20no%20specific%20legislation,%2C%201977%20(see%20below).
[33] Directive 2004/35/CE on environmental liability with regard to the prevention and remedying of environmental damage: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=LEGISSUM%3Al28120
[34] https://www.citizensinformation.ie/en/environment/environmental_law/eu_environmental_law.html#targetText=Environmental%20crime%20covers%20acts%20that,the%20environment%20and%20human%20health.&targetText=Directive%202008%2F99%2FEC%20on,adopted%20on%2028%20October%202008.
[35] http://www.eugris.info/FurtherDescription.asp?e=183&Ca=1&Cy=3&DocID=B&DocTitle=Policy_and_regulatory&T=France

2. Mapping of polluted sites in the EU testing areas of the LIFE BIOREST project

The LIFE BIOREST project's findings are a snapshot of the extent of polluted sites in Italy, Spain and France where the project has been implemented. A fragmented situation has been revealed, especially in terms of the harmonisation of criteria and available information in national and regional registers.

The most relevant polluted sites in Italy are the Sites of National Interest (Siti di Interesse Nazionale, SIN), classified according to the extent of environmental contamination, health risk and social alarm (DM 471/1999[36]). These areas are defined by Legislative Decree 152/2006[37], which indicates the site characteristics, the concentration and the hazards of the present pollutants and the environmental impact at both sanitary and environmental levels. The national remediation program, established by the Ministry of the Environment, periodically provides an updated framework on the contamination status of SINs. Currently, 41 SINs (updated as of 2018) have been classified, including the Fidenza site where the testing activities of the LIFE BIOREST project take place.

SINs include disused industrial sites, where conversion activities are ongoing, subject to accidents involving the spillage of chemical pollutants and areas subject to uncontrolled disposal of even hazardous waste. Almost all the 20 Italian regions host at least one SIN, except Molise. The contaminated soil occupies an overall area of 51,403.5 hectares, equivalent to about three times the area of the Metropolitan City of Milan. The characterisation plans have so far concerned 57.3% of the total perimeter areas (29,453.9 hectares), and in 94.7% of cases they have been implemented. There are 1,574.5 hectares affected by prevention measures, around 3% of the total perimeter, where solutions are applied to reduce the impact of toxic substances on the environment and human health.

In summary, the remediation activities have so far only concerned 12.6% of the total of the SIN areas (6,513.1 hectares, according to the last national analysis of June 2018).

Table 2. Review of areas for soil matrix concerning 41 SINs, numbers are referred to areas in hectares (ha), % of remediation is calculated on the total areas (data elaborated from SIN report of Ministry of the Environment, 2018[38]).

SIN	% remediation 2018	Prevention measures	Remediation project presented	Remediation project approved	Remediated area	Total Area
1 - Venice	15%	0	1,146	1,055	241	1,618
2 - Napoli Orientale	6%	89	174	127	50	834
3 - Gela	0%	0	120	101	4	795
4 - Priolo	8%	11	1,000	733	449	5,814
5 - Manfredonia	18%	8	67	42	38	216
6 - Brindisi	6%	0	723	692	378	5,851
7 - Taranto	8%	12	341	335	347	4,383
8 - Cengio and Saliceto	0%	0	77	77	0	77
9 - Piombino	45%	0	239	121	422	931
10 - Massa and Carrara	5%	0	46	29	5	116
11 - Casal Monferrato	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
12 - Balangero	0%	305	52	16	0	314
13 - Pieve Vergonte	0%	0	42	42	0	42
14 - Sesto San Giovanni	32%	56	215	113	82	255
15 - Pioltello-Rodano	13%	36	72	28	11	85
16 - Napoli Bagnoli Coriglio	0%	0	234	234	0	249
17 - Tito	4%	25	25	25	13	315
18 - Crotone-Cassano-Cerchiara	13%	7	150	135	69	544

[36] https://www.gazzettaufficiale.it/eli/id/1999/12/15/099G0540/sg

[37] https://www.gazzettaufficiale.it/atto/serie_generale/caricaDettaglioAtto/originario?atto.dataPubblicazioneGazzetta=2006-04-14&atto.codiceRedazionale=006G0171

[38] https://annuario.isprambiente.it/ada/downreport/html/6798

19 - Fidenza	8%	11	23	23	2	25
20 - Caffaro Torviscosa	0.49%	0	200	10	1	201
21 - Trieste	7%	0	162	124	29	435
22 - Cogoleto	0%	0	33	10	0	45
23 - Bari	0%	1	11	11	0	14,5
24 - Sulcis Iglesiente Guspinense	8%	117	1,029	922	904	19,751
25 - Biancavilla	1%	25	25	25	0	330
26 - Livorno	0%	0	206	0	0	206
27 - Terni	28%	638	6	6	181	655
28 - Emarese	0%	15	16	16	0	23
29 - Trento Nord	0%	0	11	11	0	24
30 - Brescia	1%	0	43	43	4	262
31 - Broni	1%	13.5	9.8	9.8	0.1	15
32 - Falconara Marittima	0%	0	3	3	0	101
33 - Serravalle Scrivia	0%	0	7	7	0	74
34 - Lakes of Mantua	3%	0	188	63	19	614
35 - Orbetello	0%	0	0	0	0	204
36 - Porto Torres	12%	0	944	157	226	1,874
37 - Val Basento	88%	96	30	23	2,925	3,330
38 - Milazzo	20%	59	110	110	111	549
39 - Bussi sul Tirino	1%	50	12	0	2	232
40 - Sacco River Basin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
41 - Bologna Officina Grandi Riparazioni ETR	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
TOTAL	13%	1,574.5	7,791.8	5,478.8	6,513.1	51,403.5

The leading causes of contamination in 66% of the SINs are related to industrial activities (46%) and former abandoned industrial areas (20%). 12% of SINs are made up of asbestos former extraction areas: Casal Monferrato, Broni, Emarese and the Balianto Amiantifera in Piedmont, the largest asbestos mine in Europe with a high risk of onset of pulmonary oncological diseases. 10% of SINs are represented by harbour areas (Taranto and Venice, Falconara Marittima and Trieste) strongly polluted by heavy metals and hydrocarbons. Landfills (5%) and areas of complex industrial and mining activity (7%) are also included and present a variety of different contaminants (Val Basento, Crotone-Cassano-Cerchiara).

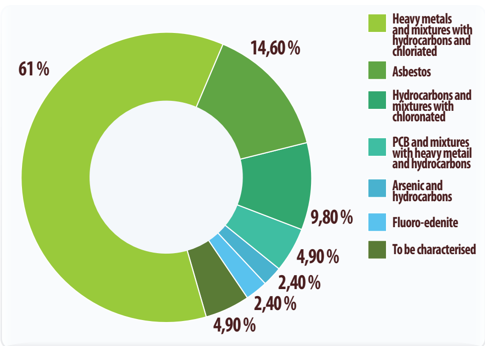


Figure 2 SIN contamination for typology of prevalent pollutants.

The chemical analysis of SINs showed a predominance of heavy metals, chlorinated compounds, hydrocarbons, pesticides and herbicides which toghether represent 61% of the total contaminants (**Fig. 2**). The most widespread pollutants are asbestos (in 14.6% of SINs), hydrocarbons (9.8%), polychlorinated biphenyls (4.9%), arsenic (2.4%) and fluoroedenite (2.4%). Hydrocarbons are present in 53.7% of SINs. The sites of Gela, Fidenza, Lakes of Mantua, Val Basento and Sulcis Iglesiente Guspinese display major hydrocarbon contamination. This is data processed starting from the assessment of the pollution sources recorded within the technical characterization reports carried out at the SINs and from the information made available by the regional registry offices.

As regards the mapping of soil contamination in Spain, the table below records the total number of contaminated sites inventoried in the Andalusia Region, divided by provinces: Córdoba is the highest (5,676 sites), followed by Sevilla (4,986) and Granada (4,238).

Table 3. Total number of contaminated sites inventoried in the 8 provinces of the Andalusia Region. (Data from http://descargasrediam.cica.es/)

	Almería	Cádiz	Córdoba	Granada	Huelva	Jaén	Málaga	Sevilla	TOTAL
N. emplazamientos inventariados	2,841	2,404	5,676	4,238	1,580	2,540	4,712	4,986	**29,277**

Detailed information about the typology of the pollution is missing. However, a map at national level from the Ministry of Energy (**Fig. 3**) shows a wide distribution of areas with concessions for hydrocarbons. The map indeed shows several hotspots for hydrocarbon-related activities in Spain, with a very high concentration of sareas in Andalusia. This outcome is particularly relevant for the LIFE BIOREST project's activities given the location of CSIC units in this region, that can allow an improved outreach of the protocols to be developed in the next steps of the project.

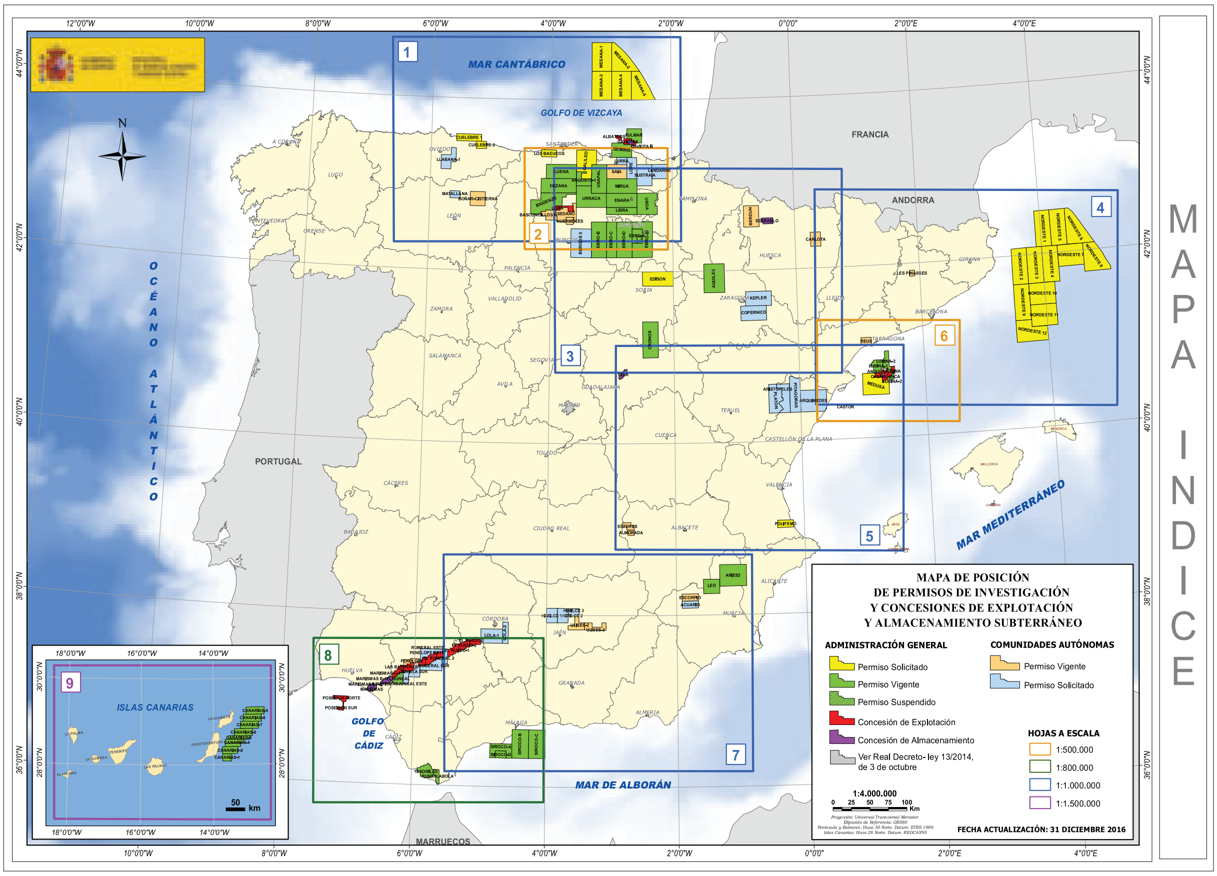
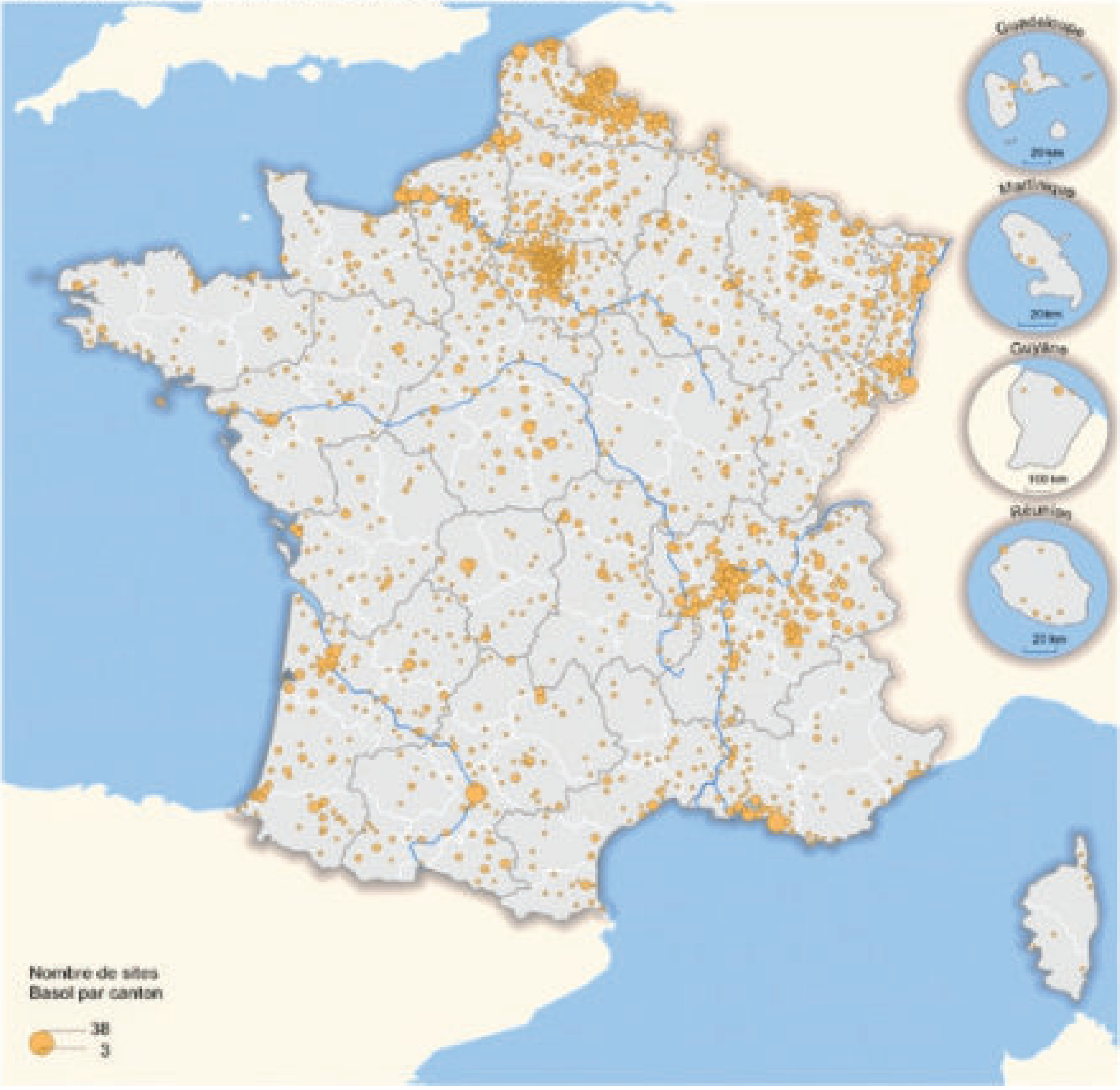


Figure 3 Map of Spain showing the total concession and licences for hydrocarbons.

An inventory of soil contamination in France registers 4,142 sites (updated in 2012) distributed across the national territory. Hydrocarbons are the main cause of pollution, affecting 33% of both contaminated soils and waters. However, this percentage refer only to linear hydrocarbons: by including aromatic hydrocarbons, chlorinated hydrocarbons and BTEX, the numbers rise to 67% for both soils and water sites.

Les sites et sols pollués début 2012
(sites sur lesquels l'état a enterpris des actions de remédiation au 16 janvier 2012)



Note: sites de la base de donnèes Basol faisant l'objet d'action de surveillance ou de réhabilitation.
Source: Medde, DGPR (Basol au 16 janvier 2012), 2012. Traitements: SOeS, 2012

Figure 4 Map of France showing the location of polluted sites.

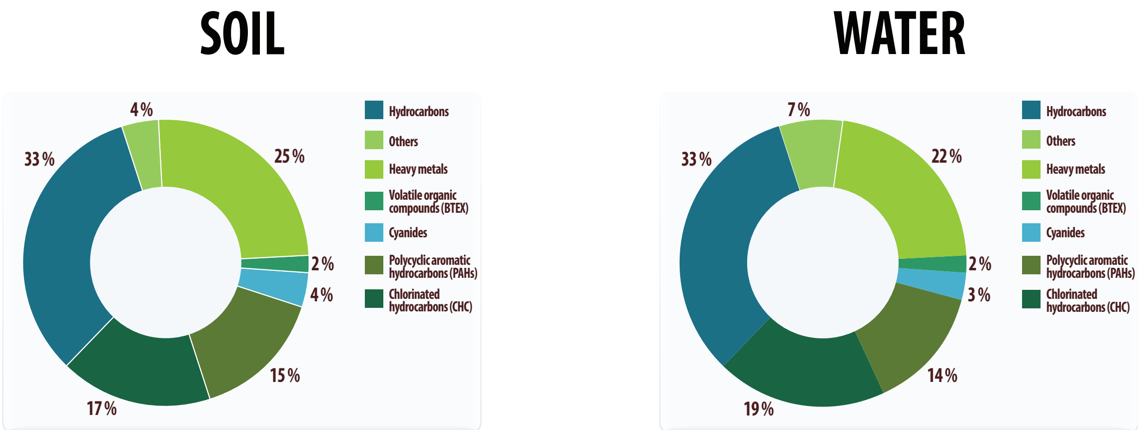


Figure 5 Classification of polluted sites according to the chemical classes: soils are reported on the left, water on the right.

3. Bioremediation techniques

The nutritional versatility of microorganisms can be exploited for the process of pollutant biodegradation, or as it is otherwise known, bioremediation. This process is based on the ability of certain microorganisms to convert, modify and use toxic pollutants for their development and growth (Tang et al., 2007). Bioremediation is a microbiological well-organised procedural activity applied to break down or transform contaminants to less toxic or non-toxic compounds (Abatenh et al., 2017). When deciding whether to use the bioremediation technique the following should be considered: the nature of pollutants (e.g. agrochemicals, chlorinated compounds, hydrocarbons, heavy metals), the depth of pollution, the concentrations of contaminants, the physico-chemical properties of the soil to be treated, and the cost of the treatment and the environmental policies.

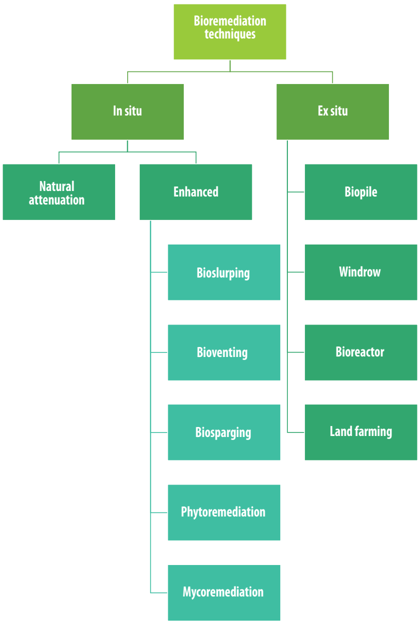


Figure 6 In situ nad ex situ soil remediation techniques.

Microorganisms are an eco-friendly alternative to the conventional remediation processes and a promising valuable genetic material to solve environmental threats.

Bioremediation can be carried out through in situ or ex situ techniques, depending on several factors such as the cost, the site characteristics and the type and concentration of pollutants.

In situ bioremediation techniques include the treatment of polluted substances at the site of pollution, without any excavation. They represent a less expensive alternative to *ex* situ techniques, since the cost for the excavation process is not required and the dispersion of contaminants is negligible. *In* situ bioremediation techniques have been successfully applied in the treatment o soil for chlorinated solvents, dyes, heavy metals, and hydrocarbons (Azubuike et al., 2016; Frascari et al., 2015; Kim et al., 2014).
Ex situ bioremediation techniques entails the excavation of pollutants from polluted sites and the subsequent transport to another site to be subjected to the treatment. The achievement of the remediation objectives in terms of competitive costs and times depends upon a wide series of parameters of optimization, whose monitoring requires a great application expertise (Sofo, 2010).

3.1 In situ

Among the in situ bioremediation techniques, two main categories can be distinguished:

Natural attenuation: the contaminants are left on site allowing the natural occurring processes to clean up the site. The succes of natural attenuation success depends on the subsurface geology, hydrology and microbiology and it is subject to hydrological changes, taking a considerable period of time to remove soil contaminants. It is mainly used for BTEX and chlorinated hydrocarbons (Mulligan, 2004).

Enhanced bioremediation: fungi, bacteria and plant nutrients (oxygen, nitrates) are added in order to accelerate the natural biodegradation process. These techniques have been successfully applied to remediate soils and groundwater contaminated with fuel, volatile and semivolatile organic compounds (VOCs and SVOCs), perchlorate, and pesticides.[39] The enhanced bioremediation techniques include different alternatives, which are listed below.

Bioventing: also called Bio-enhanced Soil Venting, is an in situ technique based on the natural stimulation of the normal biological activity present in the soil with the injection of oxygen through an air flow. Air is directly injected at low rates through one or more wells connected to vacuum pumps, which ensure the forced circulation of air into the contaminated unsaturated soil. When the soil is highly polluted, obstruction of the soil pores can occur, with subsequent reduction of the oxygen levels. To overcome this kind of problem, different strategies have been developed, such as the increase of oxygen levels through pulsed air injections[40]. The advantages are the low costs of plant realization and management, at the expense of the time required, which varies from some months to years. However, it can increase the normal soil biodegradation ability up to 40 times[41].

[39] FRTR - Remediation Technologies Screening Matrix and Reference Guide, Version 4.0:
https://frtr.gov/matrix2/section4/4-2.html#targetText=Description%3A,them%20to%20innocuous%20end%20products.
[40] https://www.crccare.com/files/dmfile/CTechguide_Bioremediation_Rev0.pdf
[41] http://www.arpa.umbria.it/Resources/docs/micron%2025/micron_25_45.pdf

Bioslurping: is a technique that applies vacuum-enhanced dewatering techniques to the remediation of hydrocarbon-polluted sites, through the combination of extraction of vapor from the soil and bioventing by indirect oxygen supply and subsequent stimulation of the biodegradation of the contaminant that is present (Gidakaros et al., 2007). This system uses a "slurp" that elongates into the layer, which draws up liquids (free products and soil gas) and other substances. This mechanism allows the recovery of free compounds, volatile or semi-volatile organic compounds and light non-aqueous phase liquids (LNAPLs) (Kim et al., 2014). Although bioslurping is almost never used for soils with low permeability, it helps to save costs due to the lower amount of groundwater resulting from the operation, minimising storage, treatment and disposal costs (Philip et al, 2005).

Biosparging: is a technique analogous to bioventing, in which there is an air injection into the soil subsurface. This action is able to stimulate microbial activities in order to allow pollutant removal from contaminated sites. In biosparging, air (or oxygen) and nutrients are injected into the saturated zone to increase the biological activity of the microorganisms, thus promoting the rise of volatile organic compounds towards the unsaturated area to increase biodegradation.

There are two main factors that regulate the efficiency: the pollutant's biodegradability and the soil permeability that regulates the bioavailability of the contaminants to the microorganisms. It is often applied to treat aquifers contaminated with petroleum products but also with benzene, toluene, ethylbenzene, xylene (BTEX) (Kao et al., 2008).

Phytoremediation: technique is based on the use of plants in polluted areas to reduce the concentration of contaminants. Organic compounds are eliminated by degradation, rhizoremediation, stabilisation and volatilisation, whereas heavy metals are removed by transformation, extraction and sequestration (Azubuike et al., 2016).
The following mechanisms are used in the process of phytoremediation:

- enhanced rhizosphere biodegradation: the release of natural substances from plant roots to supply nutrients to microorganisms which enhance biological activity;
- phytoaccumulation: the uptake of contaminants by plant roots and transfer to the plant's shoots and leaves;
- photodegradation: metabolism of contaminants in plant tissues;
- phytostabilisation: the production of chemicals by the plant that immobilises contaminants at the interface between the roots and soil (Azubuike et al., 2016).

Microbial Remediation: bacteria and/or fungi are used to transform contaminants in the soil and groundwater. Microorganisms are also capable of releasing aspecific oxidative enzymes that can break down the organic contaminants. The type of microbes used is closely related to the temperature, soil pH and the availability of oxygen. Microorganisms are capable of degrading petroleum hydrocarbons and chlorinated compounds but they can also accumulate heavy metals inside the cell wall.

3.2 Ex situ

Biopiles: is an ex situ application of a remediation technique that uses the soil microorganisms in the soil to remove contamination from soil.

Usually, this technique is applied to petroleum hydrocarbon impacted soils, contaminated by petroleum hydrocarbons, where excavation is carried out and subsequently the soil is mixed with soil amendments, forming compost piles to allow the microorganism to enhance the degradation process. The lighter petroleum products tend to evaporate from the pile due to aeration, but the medium and heavy petroleum hydrocarbons are degraded aerobically. To make the processes more effective and productive, some parameters and features can be controlled such as temperature, oxygen, pH, aeration and nutrients.

The biopile is one of the most used ex situ bioremediation techniques mainly for cost-effectiveness, even though many parameters have to be controlled to establish effective biodegradation (Whelan et al., 2015).

Windrow: is a specific ex situ remediation technique based on periodic turning of piled contaminated soil to increase bioremediation by enhancing the degradation activity of microorganisms. The periodic turning of contaminated soil, together with the addition of water is able to increase the aeration and to even out the distribution of pollutants, nutrients and microbial degradative activities. Bioremediation can be sped up as a result (Barr, 2002). However, it may not be the best option to choose in the presence of volatile compounds. The use of windrow treatment has been indeed involved in CH_4 (greenhouse gas) release due to the development of an anaerobic zone within the piled contamined soil, which usually occurs following reduced aeration (Hobson et al., 2005).

Bioreactor: When the material is removed from the environment, it can be put into bioreactors, which are large vessels in which the contaminated material can be monitored and the conditions for bioremediation can be set. Different operating modes of bioreactors exist, including batch, fed-batch, sequencing batch, continuous and multi-stage. In a bioreactors, it is possible to control the mixing rate, temperature, pH, and nutrient levels, supporting the natural process of microorganisms by mimicking and maintaining their natural environment to provide the optimal growth conditions (Azubuike et al., 2016).

Land Farming: is represents an engineered bioremediation system useful for treating remote sites due to the minimal equipment required, which generally uses passive aeration by tilling the contaminated soil to reduce contaminant levels (EPA 2014). Land farming bioremediation is considered one of the most straightforward techniques, easy to plan and implement, making in possible to treat a large area of contaminated soil with a low environmental and energy impact (Azubuike et al., 2016).

4. Soil bioremediation in three steps: the Life Biorest method

The LIFE BIOREST project involves three European countries - Italy, France and Spain - and it has been developed to demonstrate the efficiency and cost-effectiveness of the bioremediation approach for contaminated soil by polycyclic aromatic hydrocarbons (PAHs), benzene, toluene, ethylbenzene, xylene (BTEX) and long-chain alkanes thus contributing to the scientific knowledge needed for the development of European environmental and soil protection policy. The project has overseen collaboration among Consorzio Italbiotec, as the project coordinator, Actygea Srl, Agenzia regionale per la Prevenzione, l'Ambiente e l'Energia - ARPAE, University of Torino, Università Cattolica del Sacro Cuore, Agencia Estatal Consejo Superior de Investigaciones Científicas (Spain) and SATT- SAYENS (France).

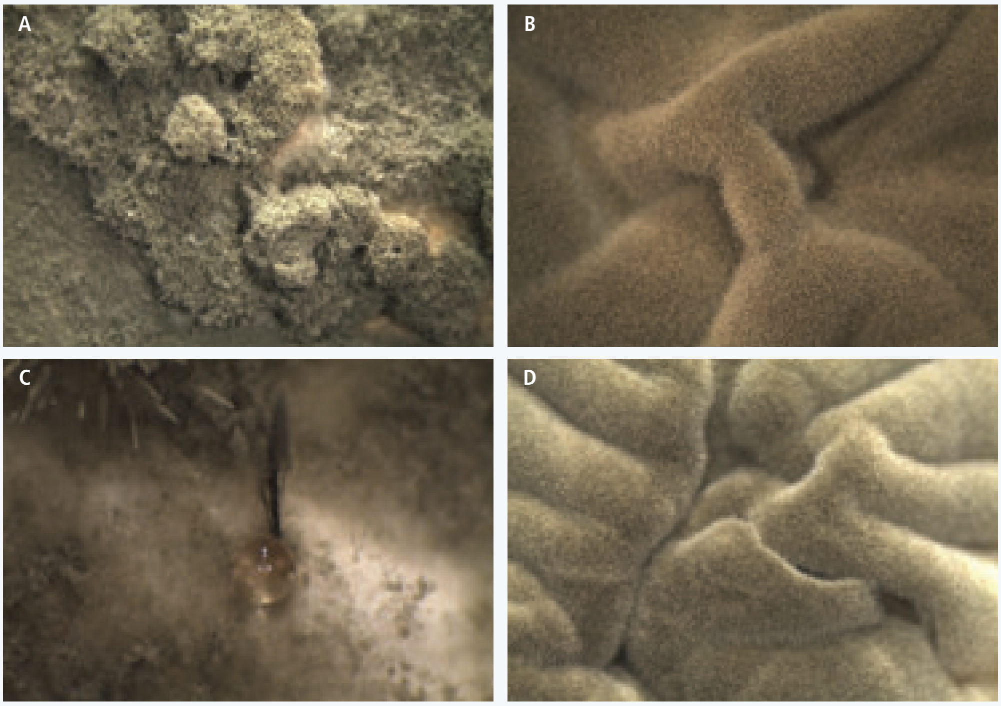
The developed biotechnology approach is based on microorganisms, agricultural by-products and plants, with the aim to re-vegetate, restore the contaminated soil and return it to public use. The LIFE BIOREST project's experimental activities aim to validate a sustainable Bioremediation Model able to treat PAHs, BTEX and alkanes which are, together with heavy metal, 45% of the total contaminants in Europe. This is true also on the Fidenza site (in Emilia-Romagna, Italy), where the bioremediation takes place.

Experimental activities started in July 2016, in the area of "ex-Carbochimica", a Site of National Interest (SIN) in Fidenza, thanks to the support of the Municipality that has provided infrastructure and spaces already affected by other reclamation activities.

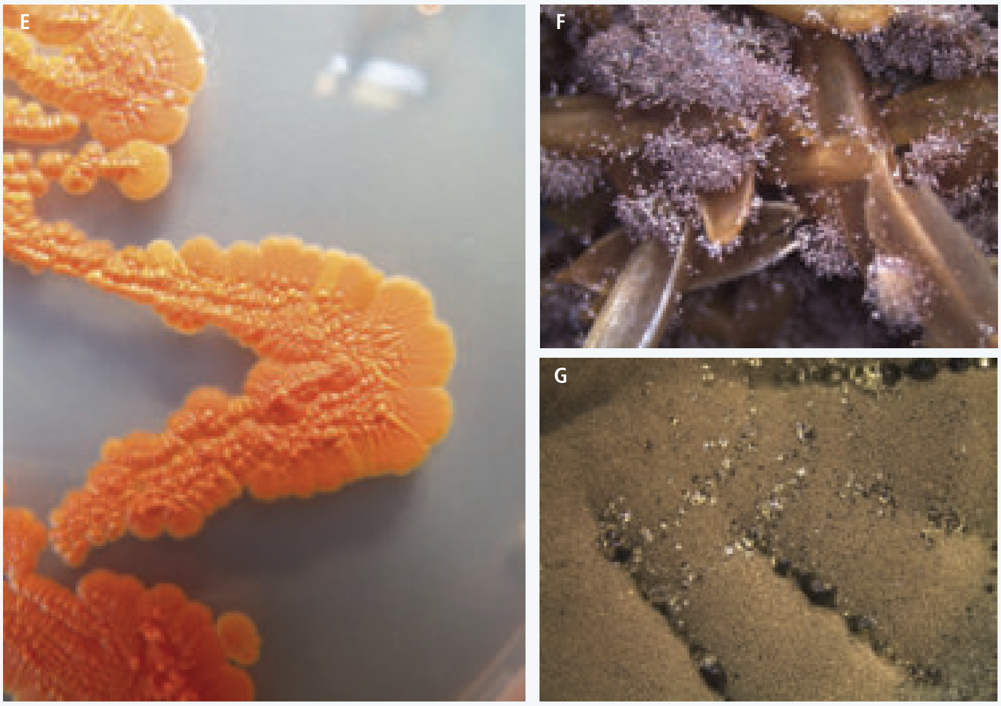
4.1 Optimisation of soil bioremediation

The first step of the LIFE BIOREST project was to concentrate its efforts in the selection of microbial strains that have been further exploited for the treatment of the contaminants. The approach of bioremediation has foreseen the use of autochthonous microorganisms, that naturally populate the contaminated site; allochthonous strains were not selected since their effects could be unpredictable when introduced into the ecosystem. Even though the contaminated soil is compromised compared to clean soil, its living biome may include microorganisms strongly adapted to extreme conditions.

Through preliminary studies, the vitality of the soil collected on the Fidenza site was assessed. The bacterial and fungal community are less abundant than those of a non-anthropised soil, but they were found to populate this ecological niche: 10^7 and 10^4 cfu/100g of bacteria and fungi respectively were detected. The contaminants were able to create an extreme environment, but nature learnt to adapt to these harsh conditions. For this reason, the project interest was focused on the selection of those strains that are able to survive in extreme conditions and also capable of colonising the contaminated soil. The application of a selective pressure during the isolation procedure allowed the isolation of 309 fungi and 256 bacteria belonging to a wide biodiversity (e.g. 78 fungal and 46 bacterial taxa). This heterogeneous microbial richness was narrowed down, identifying those few strains that could be used to treat the soil. The evaluation of this huge number of strains and variables needed to advance the traditional analytical methods that are too long and too costly. An innovative miniaturising technique was originally developed and optimised to test the growth rate of microorganisms in the presence of target pollutants (pyrene, phenanthrene, naphthalene, benzene, paraffine oil, heptadecane). Several strains showed the ability to efficiently utilise at least one of the pollutants a sole carbon source, highlighting their capability to exploit complex sources of nourishment as well as simple and bioavailable ones (e.g. glucose). Moreover, many strains were also capable of producing surfactants, that can enhance the bioavailability of organic pollutants in soil. This project helped to reveal a hidden microbial richness that could be applied to many different biotechnological fields. These results confirmed the adaptation skills triggered by the extremely toxic environment of Fidenza soil that drove microorganisms to develop a unique metabolic pathway. More than 30 bacteria and fungi were selected. Microorganisms were considered not only for their ability to degrade high impact pollutants but also for their ability to produce biosurfactants, bioemulsifiers and enzymes. Indeed, these compounds could be relevant for some aspects of the biodegradation of the pollutants and also for the stimulation of other relevant microorganisms. The best 5 performing microbial consortia were applied to a larger soil volume: bio-augmentation led to a better degradation than controls. The consortium most capable of maximising the degradation of long-chain hydrocarbons and PAHs, reducing the soil toxicity the inner soil toxicity, was chosen.



Panel 1 (A) Penicillium crustosum; (B) Cladosporium subuliforme; (C) Cephalotrichum stemonitis; (D) Cladosporium



Panel 2 (E) Rhodococcus ruber; (F) Aspergillus terreus; (G) Cladosporium perangustum

4.2 Industrial-scale biomass production

The main goal of the LIFE BIOREST project was been to scale the proof of concept obtained in microcosm and mesocosm experiments, on a real biopile with the contaminated Fidenza site. The microbial strains were used for the bioaugmentation of a portion of a biopile of approximately 530 tons, in collaboration with Fidenza Municipality. A considerable amount of microorganisms were needed to reach this goal. For this reason, the second step of the LIFE BIOREST project has foreseen the achievement of economic and practical conditions for the growth of the most suitable microorganisms, allowing the technique to be applied in the field. The accomplishment of cost-effective production, the production of microorganisms in multi-purpose fermentation plants and the adaptation of the available equipment and logistics to the production of fungi were the main objectives of the second step. Thanks to the availability of a system for selecting fermentation media for industrial production (the proprietary database ActyMedDat), the cost of the fermentation products was reduced to less than € 10 for the production of microorganisms required for the treatment of 1 ton of soil. During the fermentation, vegetable waste materials (like cellulose, rice husk, spent vegetable oil) were used. The results made it possible to identify the best microorganisms not only for their potential for biodegradation but also for the possibility to produce them on a large scale and at a low cost. The upscaled production of microorganisms was achieved and allowed the in-field application of the bioremediation process. A general protocol of scaling-up was developed and is ready to be tested in other sites that are to be remediated.

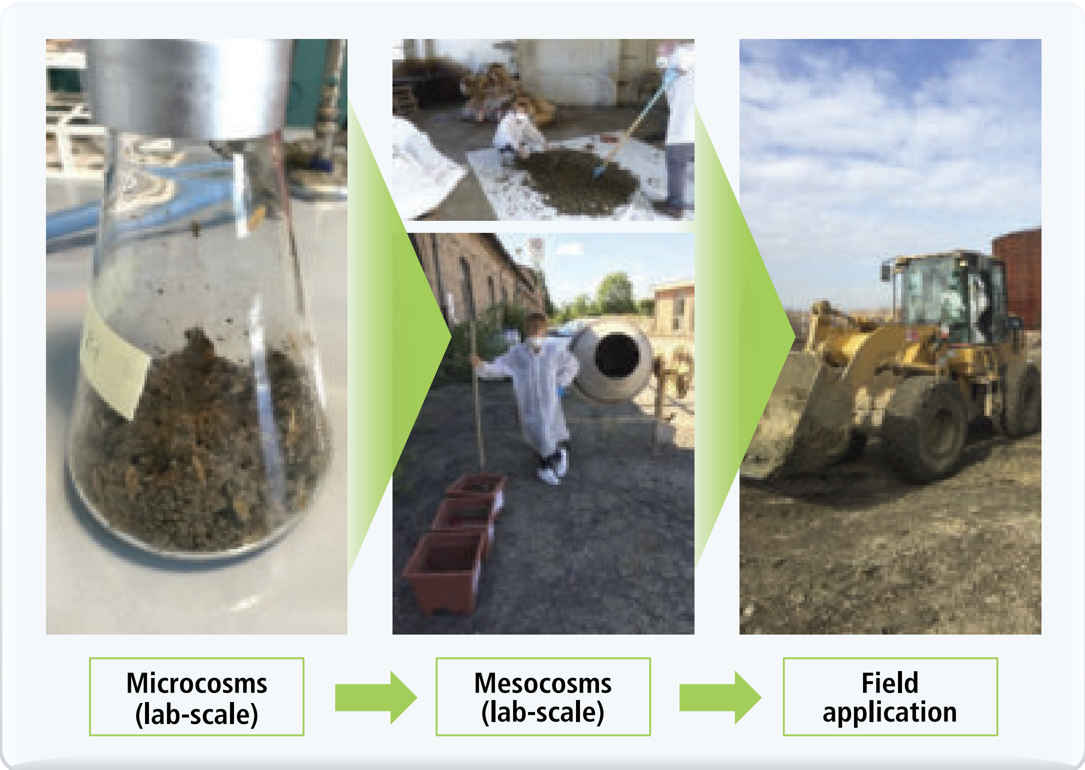


Figure 7 The upscaling of the process

4.3 In situ bioremediation and revegetation

In the third step, the LIFE BIOREST project has focused on the biopile preparation with the best microbial consortium previously selected. The biopile was prepared with 530 tons of contaminated soil and the bacteria and fungi belonging to the best performing consortium were applied to the soil. The soil was then shaped into a standard 3m high biopile. The biopile was continuously aerated and the humidity of the soil was controlled all throughout the incubation period. The bioaugmented fraction showed a faster pollutant removal. For instance, after 60 days, the total concentration of hydrocarbons was unaltered in the control, but a removal of up to 40% was already achieved by the microbial treatment. The changes in the chemical composition of the soil also correlated with the whole soil toxicity. Again, the bioaugmentation led to a faster decrease in toxicity. For instance, as regards the sorghum and the luminescent bacteria *Vibrio fischeri*, the toxicity was up to 2 times lower in the presence of the microbial treated soil. In order to couple bioremediation with phytoremediation, pot experiments were carried out to select the three plant species most suitable for the revegetation after the biopile treatment. Three species were the most indicated for phytoremediation in the soil contaminated with PAHs and BTEX: Sorghum bicolor, *Trifolium pratense* and *Festuca arundinacea*.

Figure 8 Application of the produced microorganisms to the mesocosms and to the biopile

Figure 9 Biopile preparation

Figure 10 Greenhouse pot experiments

Figure 11 LIFE BIOREST revegetation area

5. Bioremediation recommendations

Soil is considered a resource, whose health maintenance is essential for promoting the basic functions of supplying essential nutrients, water, oxygen and support for plants. Soils represent a critical part of the hydrological cycle and contain large quantities of carbon which, if released into the atmosphere, can accelerate the rate of global warming and, consequently, of the climate change.

Despite the vital importance of soil in everyday life, its improper use and management such as unsustainable industrialised agriculture, the unawareness and other socioeconomic factors mainly have led to the destruction of good soils.

For this reason, a growing boost to the achievement of sustainability in all its variations has put the issue of soil contamination and protection in the spotlight in the European framework. Indeed, the 7th EAP has continued to promote the sustainable use of soil: 'land shall be managed sustainably in the Union, soil shall be adequately protected, and the remediation of contaminated sites will be well underway'[42].

Significant progress has been made during recent years in most European countries in dealing with historical site contamination and in setting targets for the management or the complete remediation of these sites. According to data from the Joint Research Centre, 650,000 contaminated sites have been registered in the inventories of the 28 Member States, where reclamation treatments have been carried out or are ongoing. More than 76,000 new sites have been registered from the previous analysis of 2014. Currently, 65,500 sites have been subjected to corrective measures (Peréz et al., 2018).

In industrialised countries, the remediation of contaminated sites has become a real issue, thanks to the development of an "ecological conscience" which has raised awareness of the gravity of the current situation.

Soil contamination management presents particular aspects linked to the high variability of cases and to the availability of economic resources, which allow the adoption of more innovative and sustainable technologies.

The remediation of soils is a complex combination of different technologies and approaches which have evolved along the years. Traditional remediation techniques are based on the transfer of the contaminated soil to landfills, which is simply relocating the pollution, making it technically limited, inefficient and poorly environmentally sustainable, especially when considering the management timescales and the environmental impacts involved. Traditional remediation techniques are still prevalent in the treatment of contaminated soils, in particular soil excavation and disposal, accounting for on average 30% of such activities. In situ and ex situ measures are applied with the same frequencies. The costs of excavating and transporting large quantities of contaminated materials for ex situ treatment makes the currently available physical methods more expensive.

However, the increase of regulatory controls of landfill operations and associated rising costs, combined with the development of innovative ex situ and in situ remediation techniques, is changing the pattern of remediation practices.

The high cost has led to an increasing interest in alternative technologies for in situ application, in particular, those based on the biological degradation ability of plants and microorganisms (Chaudhry et al., 2005).

Bioremediation offers an interesting alternative to traditional approaches, with its limitations and benefits. Fungi, bacteria and plants may evolve adaptation skills that help them to populate the contaminated ecological niche. Among the advantages of preferring bioremediation techniques there are the minimal equipment requirements and the low cost of treatment per unit volume of soil compared to other remediation technologies. Furthermore, the importance of soil as a non-renewable resource which must be protected and preserved, makes bioremediation a more sustainable option with long-term benefits, even though it takes more time than other alternatives such as landfilling and incineration. The most significant benefit of using a bioremediation process is its contribution to the environment since it uses nature to fix nature. Bioremediation also has limitations in its application, since controlling of volatile organic compounds (VOCs) may be difficult when an ex situ process is used, and the conditions - pH, temperature, oxygen - have to be controlled and monitored to maintain the microorganism viability and activity.

Besides the current knowledge and application, bioremediation has always covered a consistent part of the soil quality restoration. Indeed, microorganisms (the engine of bioremediation) evolve, act and remediate invisibly from the beginning of the pollution process. Only in recent years have we acquired increasing consciousness of the underestimated work of those invisible machines. On the other side, we have also purchased the awareness on how to fuel our

[42] The 7th Environment Action Programme (EAP) - 2013: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32013D1386

microbial machines to run them faster and to allow them to reach niches which are incompatible with their work. The target of the LIFE BIOREST project was to supply fuel to indigenous microorganisms in their action against pollutants in the Fidenza site. The project has reproduced at an accelerated pace the natural evolution of a perturbed environment struggling for a way back to nature. The application of the indigenous microorganisms has allowed a faster degradation of the target pollutants.

Once the bioremediation process performed in biopiles is sufficiently finished, the forced colonisation of the soil with vegetables has further pushes the restoration of a healthy environment. Indeed, the mechanical action of plant roots has generated "soil motorways" along which microorganisms have continued their degradation of pollutants.

It has to be pointed out that the approach used in the LIFE BIOREST project is fully compatible with the biopile (tested within this project) or landfarming technologies (not tested within this project). Therefore, the economic impact on the remediation process itself tends to be marginal, with only the costs of production of microorganisms to be added (calculated in the range of € 10-50 per ton of soil to be treated).

Although the LIFE BIOREST approach is easily translatable to other polluted sites, one of the most significant problems with developing cost information is that costs reported under a set of conditions at one site are very difficult to extrapolate to other sites. Like technology performance, technology costs are sensitive to site-specific geologic, geochemical, and contaminant conditions, especially for in situ technologies. The remediation of contaminated soils with a standard biopile approach (such as that used initially in the Fidenza site) has a cost which is deeply influenced by the type of pollutants present and the containment requirements to be applied (the cost could range approximately from € 200-1,000 per ton of soil).

However, excluding chlorinated pollutants, the application of microorganisms through the LIFE BIOREST approach is universal and independent from the type of contaminant present in the soil. In conclusion, being conscious of the fact that remediation of soils is an integrated approach, the potential of indigenous microorganisms and local flora is of paramount importance in the restoration of soil quality of soil and impacts marginally or even positively on the costs of remediation.

The model for soil remediation proposed by the LIFE BIOREST project has enclosed an understanding of the profound importance of soil for human life, aiming to educate the public about the crucial role of land. For this reason, many activities have been organised for students, involving more than 1,000 of them in research and communication activities regarding soil prevention and treatment, and encouraging them to become active in raising public awareness.

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