LIFE Project Number

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Final Report

Covering the project activities from 01/07/2016 to 31/12/2019

Reporting Date

30/06/2020

LIFE PROJECT NAME

LIFE BIOREST

Bioremediation and revegetation to restore the public use of contaminated land

Data Project

Project location:	Italy
Project start date:	01/07/2016
Project end date:	30/06/2019 **Extension date:** 31/12/2019
Total budget:	€ 1.710.267
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(%) of eligible costs:	

Data Beneficiary

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1. Table of contents

2. List of key-words and abbreviations

AB	Associated Beneficiary
ACTY	Actygea Srl
ARPA	Agenzia Regionale per la Prevenzione, l'Ambiente e l'Energia dell'Emilia- Romagna
BTEX	Benzene, Toluene, Ethylbenzene, Xylene
CB	Coordinating Beneficiary
cfu	Colony Forming Units
CSIC	Agencia Estatal Consejo Superior de Investigaciones Científicas
DC	Dissemination Committee
HC	Hydrocarbons
ITB	Consorzio Italbiotec
MCB	Mater Cell Bank
OB	Objectives
PAH	Polycyclic Aromatic Hydrocarbons
PMV (%)	Packed Mycelium in Volume
R	Results
UCSC	Università Cattolica del Sacro Cuore
UNITO	Università degli Studi di Torino
WCB	Working Cell Bank
SATT Sayens	SATT Sayens (ex Satt Grand-EST – Welience WEL)

3. Executive Summary

LIFE BIOREST (www.lifebiorest.com) is a LIFE project involving three European countries (Italy, France and Spain) conceived to demonstrate the **efficiency and cost-effectiveness** of **bioremediation approach** for polluted soil by PAHs, BTEX and long-chain alkanes contributing to the scientific knowledge needed for the development of European environmental and soil protection policy.
The project foresees the collaboration among Consorzio Italbiotec, Coordinating Beneficiary (CB), and the Associated Beneficiaries (AB) Actygea Srl, Agenzia regionale per la Prevenzione, l'Ambiente e l'Energia - ARPAE, University of Turin, Università Cattolica del Sacro Cuore, Agencia Estatal Consjeio Superior de Investigaciones Cientificas (Spain) and SATT Sayens (France).

The biotechnology approach is based on microorganisms, agricultural by-products and plants, aiming to re-vegetate and restore contaminated soil and its return to public use. LIFE BIOREST experimental activities are focused on validating 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 kind of contamination is true also in Fidenza's site (Emilia Romagna, Italy), where the bioremediation project takes place. Experimental activities started in July 2016, in the area of "ex-Carbochimica", Site of National Interest (SIN) in Fidenza, thanks to the support of the Municipality that is providing infrastructure and spaces already affected by other reclamation activities. **The demonstration activities lasted for 42 months and ended in December 2019, with a global of the bioremediation approach validation at the industrial level.**
LIFE BIOREST ensured the achievement of several results that enhanced the effectiveness of *in-situ* bioremediation treatments, and the dissemination of their advantages to an audience of stakeholders, citizens of sites neighbouring areas, industrial groups and students.

Specific LIFE BIOREST objectives aim at:

- Optimize protocols and **guidelines for bioremediation** potentially applied in other scenarios.
- Demonstrate how the adopted approach can achieve a reduction of contaminants concentration, complying to the threshold limits for **residential and public land use**.
- Revegetate the decontaminated soil, restore its ecological functions, and returning it to public use.
- Exploit the microbiological remediation of contaminated sites as a means to **spread knowledge** and consciousness about environmental pollution, risk assessment, biodiversity, ecotoxicology, soil ecology, and bioeconomy.
- Disseminate the clear societal benefits of addressing the issue of **soil contamination** at the European level (supporting the Seventh Environment Action Programme).

Project main project **results** are:

- Optimised and tailored protocols for the bioremediation of the test site, based on a combination of microbial consortia with improved degradation abilities towards alkanes, PAHs and BTEX.
- Validate the scaling-up method for the production of microorganisms able to guarantee efficient bioremediation of a demonstration area of 400 m^3 in the site "ex-Carbochimica" of Fidenza.
- Draw up Guidelines to exploit indigenous microorganisms for the remediation of contaminated land to reduce soil pollutants concentrations, compatible with residential or public use.
- Re-establishment ecological functions of the treated soil, showing the possibility of cultivating different plant species and production of chemical, microbiological and eco-toxicological indicators of soil quality restoration.

4. Introduction

Soil degradation is a serious problem all around Europe. It is usually driven or exacerbated by human activities such as inadequate agricultural or forest practices, industrial activities, waste disposal, oil spills, urban and industrial proliferation and construction works.
The main negative impacts of soil degradation are loss of fertility and biodiversity, reduced water holding capacities, impairments of biogeochemical cycles and reduced resilience and buffer capacities.
It is estimated that there are around three EU member states millions of potentially contaminated sites. Estimates show that more than 10% (more than 340,000 sites) are contaminated and need interventions, but this percentage is overgrowing, and it is estimated that the total number of sites contaminated that needs reclamation may become 50% by 2025.
The contamination found is attributed to commercial and industrial activities and treatment of waste. The most responsible industries are the metallurgical ones, the chemical poles, thermoelectric power plants and refineries and common causes of soil contamination are related to tank spills.
About 37% of sites are contaminated from heavy metals (***Fig. 1***), 34% by mineral oils, 13% by polycyclic hydrocarbons aromatic (PAHs), 6% by mixtures of benzene, toluene, ethylbenzene and xylene (BTEX), 4% by phenols and 2.4% by organic polychlorinated (Van Liedekerke M, 2014).

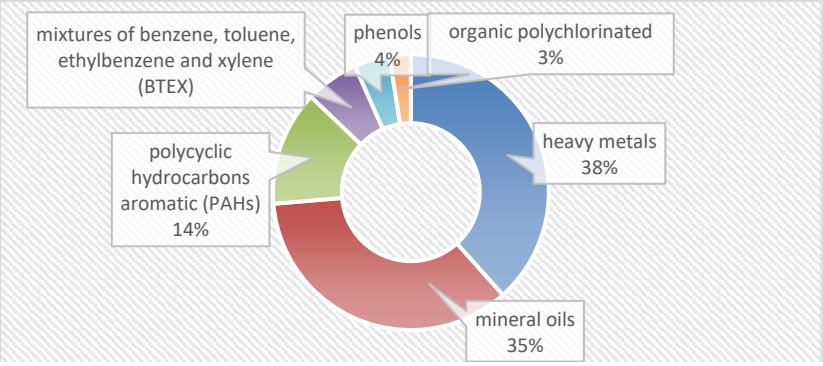

Fig. 1 *Most common contaminants in Europe*

Up to the present, the most common remediation technique has been the excavation of contaminated soil and its disposal as landfill (sometimes referred to as 'dig and dump'). However, increasing regulatory control of landfill operations and associated rising costs, combined with the development of improved ex-situ and in-situ remediation techniques, is altering the pattern of remediation practices[i]. An assessment of remediation technologies adopted in the EU indicates that ex-situ treatments are the most adopted, despite being generally expensive (Megharaj et al., 2011) and less sustainable in terms of Life Cycle Assessments (Cappuyns and Kessen, 2012), are still the most widespread ones (***Fig. 2***). Excavation of contaminated soil and disposal at landfills is indeed the most common remediation technique in several countries, reaching values of more than 50% of adopted approach in Norway, Slovakia, Italy and Lithuania and up to more than 90% in the United Kingdom. Conversely, *in situ* treatments generally account for less than 50% of adopted technology, with biological treatments constituting a minor part of it. It is however clear from the scientific literature that biological *in situ* remediation, especially for contaminants quite easily biodegradable such as petroleum oils, PAHs and BTEX, present undoubted economic and technological advantages, and it is thus necessary to better demonstrate their efficacy in technological and economic terms to promote their implementation around the EU.

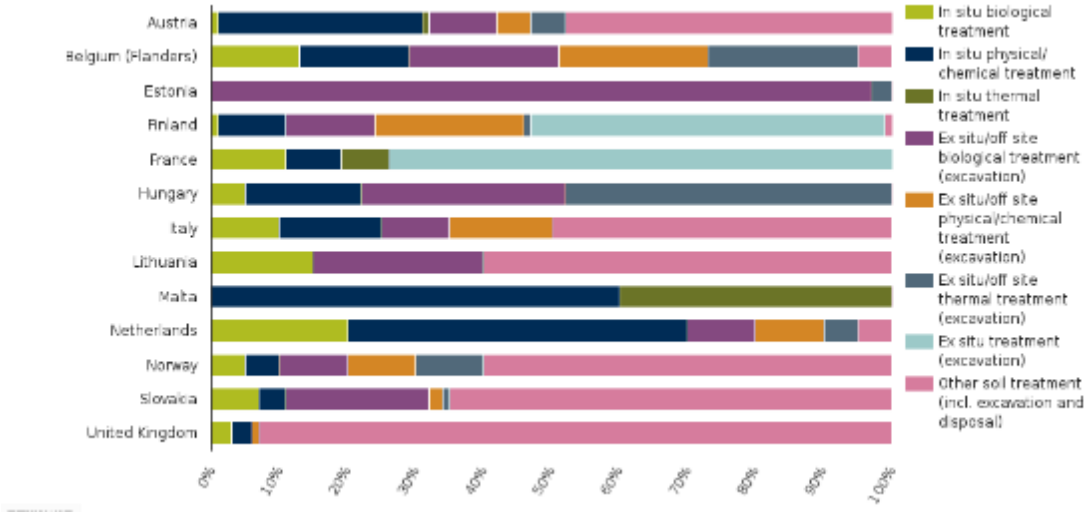

Fig. 2 *Most frequently applied remediation techniques for contaminated soil*
European Environment Agency

LIFE BIOREST project aimed to implement and demonstrate the most important and innovative aspects of in situ bioremediation of polluted soils based on the bioaugmentation with autochthons ecologically adapted bacteria and fungi. The project had to be considered at the pre-industrial level since it aimed at demonstrating the efficacy and cost-efficiency of the approach in areas of limited dimensions located within a more extensive polluted site (Municipality of Fidenza, Italy).

LIFE BIOREST activities was supported in the testing phase by the Fidenza Municipality, the owner of the contaminated area and responsible for the overall reclamation, which provided infrastructure (biopile) and facilities (soli excavators) already operating in the soil treatment. This aspect is fundamental since it respects the "polluters pay" principle adopted in the EU: the Municipality did not cause the pollution but took responsibility after acquiring the area. In the site, a biostimulation approach with biopiles is currently ongoing, and it aims at a reduction of pollutant concentrations compatible with industrial land use. LIFE BIOREST demonstrated how the bioremediation approach based on the inoculum with selected bacteria and fungi followed by revegetation could improve the remediation up to the reach of values acceptable for residential/ or public use, while the Fidenza municipality is at the moment just aiming at reaching values acceptable only for industrial use.

LIFE BIOREST ensured the achievement of several results that enhance the effectiveness of *in-situ* bioremediation treatments, and the dissemination of their advantages to an audience of stakeholders, citizens of sites neighbouring areas, industrial groups and students. Main project **Results** (**R**) are:

- Optimised and tailored protocols for the bioremediation of the test site, based on a combination of microbial consortia with improved degradation abilities towards alkanes, PAHs and BTEX
- Scale-up of the production of microorganisms for the treatment of polluted sites able to guarantee efficient bioremediation of a defined demonstration area of about 400 m^3 in the site "Ex-Carbochimica" of Fidenza
- Elaboration of Guidelines to exploit indigenous microorganisms for the remediation of contaminated land to reduce soil pollutants concentrations compatible with public use
- Re-establishment of vegetation on the treated soil, showing the possibility of cultivating different plant species and production of chemical, microbiological and eco-toxicological indicators of soil quality restoration
- Increase citizen awareness of the bioremediation benefits and the relevance of bioavailability and ecotoxicology in the risk assessment and remediation of polluted soils.

5. Administrative part

Project management process

The management structure of LIFE BIOREST was defined to ensure planning, organising and monitoring of the effort to achieve the technical objectives of the project within the constraints of time, schedule and budget, define clearly the main decision-making procedures and enable regular and efficient monitoring and performance control of the project.

During the Kick-off meeting, held July 22, 2016, the Coordinating Beneficiary (CB) ITB set up the management structure through the **Project Management Handbook** (ANNEX 1.E1).

LIFE BIOREST project partners and stakeholder organization were the primary target audience of this handbook, which is considered a working tool rather than a policy or strategic document.

LIFE BIOREST Project management handbook was structured according to the main sections of Grant Agreement and Guidelines for Applicants. Its aimed to provide tools for proper management of the project set internal and external communication strategy and ensure effective project achievements.

Fig. 3 *Project management scheme*

Management structure of LIFE BIOREST was composed by four main components (***Fig. 3***):

1. **Project Manager full-time – PM**, was responsible for: (i) Planning project management meeting and video-conference agenda, (ii) Ensure the delivery of milestone and deadlines, (iii) Overseeing the implementation of the activities, (iv) Defining the Contingency Plan, (v) Overseeing administrative management, (vi) Defining overall project reporting and performance indicator, (vii) Ensure data sharing between partners and EC, (viii) Defining the After-Life Communication Plan
2. **Project Management Staff –PMS** chaired by the PM, was composed of one representative of each partner. The PMS is responsible for: (i) Strategic and scientific orientations of the project, as well as any re-orientation in terms of the work plan, budget and partnership including measures towards defaulting partners, (ii), Ensure the relevance of the project work plan concerning the progress, (iii) Analyse performance indicators (deliverables/milestones), significant risks and all other relevant information.

The **Action Leaders – AL** were responsible for monitoring the Action progress, ensuring that the objectives of the project are performed within the strategy of the work plan. ALs are the interface between partners and the PM, ensuring optimised concerted actions and responsive scientific management. Their tasks are (i) Deliverables production due to submit to the EC offices, (ii) Ensuring the participant commitment to each Action, (iii) Present Actions progress reports (scientific and financial

reports), (iv) Identify potential risk (Contingency plan) and inform the PM of any other difficulty arising in connection with the Action.

3. Administrative and Financial Staff – AFS, was responsible for the day-to-day project administrative and meeting logistic tasks: (i) Ensures the day-to-day project management and administration in close collaboration with the PM, (ii) Set up and maintenance of all management tools required for all partners and governance bodies (i.e. project guidelines, Contingency plan, report and deliverable templates, contact and mailing lists), (iii) Financial administration (monitoring of expenses against budget allocations, consolidation of financial summary sheets, etc.) according to the contractual requirements and the certificates on financial statements when required.

4. Dissemination Committee – DC chaired by PM, is composed of one representative of each Partner. The DC is the co-decision making of the PROJECT relation to dissemination activities: (i) Dissemination Plan (DP) implementation and monitoring, (ii) Coordinate the demonstration events organization, educational visits and public events, (iii) Ensure the proper design, planning and implementation of dissemination materials, (iv) Overseeing the planning and implementation of communication tools and social network activities, (v) Analyse performance indicators and achievement of PROJECT deliverables and milestones.

The LIFE BIOREST Contingency Plan (ANNEX 2.E1) was elaborated and regularly updated to guarantee project achievements. The document allows an assessment of potential risks, therefore allowing the Consortium to take appropriate, preventive, realistic, on time and effective remedial actions.
The Partnership Agreement (ANNEX 3.E1), signed on March 13, 2017, defines the organisation between partners, the management of LIFE BIOREST, rights, obligations, IP and ethical rules within the project. The purpose of this agreement is to achieve progressive complementarities and integration among the partners and to establish sustained structures.

Main Action E1 output achieved are listed below.

- Project management Handbook
- Contingency Plan was elaborated and regularly updated. The document allows an assessment of potential risks, therefore allowing the Consortium to take appropriate, preventive, realistic, on time and effective remedial actions.
- Partnership Agreement signed on March 13, 2017, defines the organisation between partners, the management of LIFE BIOREST, rights, obligations, IP and ethical rules within the project.
- Performance indicators state-of-the-art (project beginning)
- Performance indicators final report
- Layman's report
- After-Life Communication Plan

6. Technical part

6.1 Technical progress per Action

ACTION B1 – Optimized soil bioremediation by selected degrading strains

- **STATUS OF ACTION:** COMPLETED
- **Foreseen start date:** 1/7/2016 **Actual start date**: 1/7/2016
- **Foreseen end date:** 31/12/2017 **Actual (or anticipated) end date**: 31/03/2018

- **Action leader:** UNITO
- **Partners involved in the Action:** UCSC, SATT SAYENS, ITB, ACTY, CSIC

SUB-ACTIONS DESCRIPTION

Action B1 aimed at the selection of the strains, that were further exploited for *in situ* treatment of the contaminated soil. The approach of bioaugmentation foresaw the use of autochthonous microorganisms, those that naturally populate the contaminated site; allochthonous strains were avoided being alien organisms whose effects once introduced in the ecosystem could be unpredictable. Even though the contaminated soil was certainly compromised in comparison to clean soil, its living biome included few microorganisms but actively adapted to the extreme conditions.

SUB-ACTION B1.1 - Isolation and catalytic screening of bacteria and fungi | Start: 1/9/2016 | End: 30/6/2017. Sub-Action B1.1 was dedicated to the selection of microorganisms to set up targeted bioaugmentation remediation in the Fidenza's site. Fungi and bacteria were isolated from the contaminated soil, identifying more than 350 strains. Their performances against the pollutants on interest were evaluated, and different techniques were applied to have an insight into their metabolic pathway. Many strains were capable of producing biosurfactants and oxidative enzymes. Detailed results are reported in D2.B1.

SUB-ACTION B1.2 - Optimization of bioremediation protocols | Start: 1/1/2017 | End: 30/9/2017. As reported in D3.B1, strains were selected according to different parameters as their capability to grow in the presence of pollutants as sole C source and the production of a remarkable biosurfactant. Selected consortia were inoculated in microcosms and mesocosms trials, upscaling the treated soil from 500 g up to 10 kg. Bacteria were first tested in microcosms made up to single or mixed strains. The same was done with fungi. Bacteria and fungi were then combined, inoculating up to 35 microcosms thesis. Six microbial consortia were inoculated in mesocosms trials. As indicated in D9.B1, according to the chemical, ecotoxicological and biological analysis, consortium A was the most performing one. The treated soils of all the mesocosms were then used for plant treatment.

SUB-ACTION B1.3 - Plants selection and re-vegetation conditions | Start: 1/7/2017 | End: 31/03/2018. Action B1.3 aimed at selecting plants for the *in situ* re-vegetation in Fidenza. Two experiments were performed to identify the most suitable plants' species to be used for revegetation: a germination test with polluted soil eluate and a germination test on contaminated untreated soil of Fidenza site. For both the germination tests, 14 species were tested: *Cannabis sativa, Dactylis glomerata, Festuca arundinacea, Festuca rubra, Lolium multiflorum, Lolium perenne, Lotus corniculatus, Medicago sativa, Solanum licopersicum, Sorghum bicolor, Trifolium pratense, Trifolium repens, Triticum* durum, and *Zea mays*. As reported in D7.B1, the selected species were *Festuca arundinacea, Trifolium repens*, and

Sorghum bicolor that resulted in the most indicated for phytoremediation in the soil contaminated with PAHs and BTEX.

RESULTS COMPARED WITH PLANNED OUTPUT, TIME SCHEDULE.

The microbial biodiversity of the contaminated soil was much higher than expected. Partners' efforts allowed the isolation and identification of more than 350 fungi and bacteria. They were characterized for their degradation capabilities, biosurfactants and enzyme production. The expected results foreseen by sub-Action B1.1 were achieved in time. Soil treatment was optimized, and more than 35 microcosms and 6 mesocosms consortia were run. This step required 3 additional months in the time schedule of sub-Action B1.2 that ultimately affected the end of Action B1 (postponed at the end of March 2018). The choice of the most suitable plants for the revegetation was indeed obtained within the new timeline. This change did not cause any further delay on the entire project time schedule, because the revegetation of *in situ* treated soil was not scheduled before Summer 2018.

ACTION B2 – Upscaled production of microorganisms

- **STATUS OF ACTION:** COMPLETED
- **Foreseen start date:** 1/10/2016 **Actual start date:** 1/10/2016
- **Foreseen end date:** 30/9/2018 **Actual (or anticipated) end date:** 30/9/2018

- **Action leader:** ACTY
- **Partners involved in the Action:** UCSC, UNITO, CSIC

SUB-ACTIONS DESCRIPTION

The Action B2 aimed to achieve suitable conditions (both economic and practical) for the growth of the most suitable microorganisms to be applied in mesocosm experiments and in field applications. In the frame of LIFE BIOREST, 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, biosurfactants, bioemulsifiers and enzymes could be relevant for some aspects of the biodegradation of the pollutants and also for the stimulation of other relevant microorganisms.

SUB-ACTION B2.1 - Biostimulation and optimization of growth conditions | Start: 1/10/2016 | End: 30/9/2017. The isolation of microorganisms able to degrade relevant pollutants was performed in Action B1. The microorganisms isolated in Action B1 were then tested in microcosm experiments. The goal of Action B2 was the achievement of suitable conditions for the growth of the most suitable microorganisms to be applied in mesocosm experiments and in field applications. In the considered reporting period, the conditions for the optimal growth were identified and were applied to large-scale production.

SUB-ACTION B2.2 - Large scale production of microorganisms for in situ activities | Start: 1/10/2017 | End: 30/9/2018. The main task of Sub-action B2.2 was to reach a cheap and suitable production of microorganisms, metabolites or enzymes scaled to 30-200 L volumes (revised in the midterm report for 3-30 L due to better than expected performances), to determine large-scale production criteria and to provide enough material for field testing in Action B3. The main Task was achieved through the following sub-tasks:

- Production of microorganisms and their products in a cost-effective, and environmentally sound manner after strain selection for improved performance of microorganisms and selection and improvement of the fermentation media (by use of the proprietary database ActyMedDat) (deliverables D1.B2 and D2.B2);
- Identification of the best conditions for downstream processing of microorganisms for the in-field applications (deliverables D2.B2).

As foreseen in the proposal, the production of microorganisms was achieved, and the microorganisms were applied successfully to the mesocosm experiments and the biopile. All the activities foreseen were achieved according to the timetable. The production of microorganisms did directly affect the fulfilment of the Action B3 in the preparation of the biopile, which the most relevant part of the project is itself.

RESULTS COMPARED WITH PLANNED OUTPUT, TIME SCHEDULE.

The results were entirely in line with the programmed output, better than expected results in the growth of microorganism allowed a reduction of the fermentation volumes. As a consequence, ACTY was able to avoid sub-contracting of the 200 L fermentation, and all the necessary work was organized in-house with an increase in the use of the internal resource.

ACTION B3 – In situ bioremediation and revegetation

- **STATUS OF ACTION:** COMPLETED
- **Foreseen start date:** 1/1/2018 **Actual start date:** 1/1/2018
- **Foreseen end date:** 30/6/2019 **Actual (or anticipated) end date:** 31/12/2019*
  **according to the Letter Amendment no. 3*
- **Action leader:** UCSC
- **Partners involved in the Action:** ACTY

SUB-ACTIONS DESCRIPTION

The action encompasses the final operations to be performed in the SIN of Fidenza as project finalisation. Activities started in January 2018 and finished with the end of the project.

SUB-ACTION B3.1 - *In situ* bioremediation | Start: 1/1/2018 | End: 30/3/2019. The main aim of this Sub-action was to prepare a biopile treated with the best consortium selected in Sub-action B1.2. According to the project, in situ bioremediation was carried out in Fidenza test site, taking advantage of the infrastructure and facilities already active. These facilities included the excavation of the soil to be remediated, its transportation to a covered area for its mixing with selected fungal/ bacterial inocula and carrier material (rice husk, as identified in action B1), the set-up and maintenance of a biopile that run for at least 3 months. The soil was collected from the "ex Carbochimica" area, with a presence and concentration of pollutants described in action C1.1. The biopile had a total volume of 400 m^3 (ca 350 m^3 of soil+ 50 m^3 of mixed carrier material and inocula) and covered 315 m^2 (21 m x 15 m) of field surface.

According to deliverable D2.B3, the microbial strains produced in ACTY were used for the bioaugmentation of approximately 530 tons of soil. The microbial strains were produced in ACTY according to deliverable D2.B2 and delivered to the application site according to Milestone 3.2.

The biopile was prepared as follows:

- microorganisms were mixed with a suitable amount of water (approximately 1 m^3 for 200 tons of soil)
- the microorganisms were applied to the polluted soil (in batches of 10 tons excavated from the polluted site) by use of a pump for liquids
- soil, microorganisms, amendment and nutrients were mixed with an excavator
- the prepared soil was delivered to the biopile site and arranged according to the principles of a standard aerated biopile

SUB-ACTION B3.2 - Revegetation and restoration of soil ecological functions | Start: 1/7/2018 | End: 31/12/2019. The aim of this sub-activity was the preparation of a demonstration area (provided by Fidenza Municipality in a formal agreement) where the soil treated in the biopile was moved and planted with selected species. The re-vegetation was performed on the soil remediated in the biopile using the plant species selected during the sub-action B1.3 activity.

During the month of March 2018, in agreement with the Municipality of Fidenza, the LIFE BIOREST "revegetation area" was identified. A revegetation project has been developed taking into consideration the soil treatment with the phytoremediation method and also the opportunity to provide a permanent demonstration area—the revegetated site host public visits to disseminate the effectiveness of the bioremediation method. Due to the uneven decontamination of the biopile soil, the start of revegetation in autumn or winter could be hindered by climatic conditions able to affect sowing and germination of the selected plants.

For this reason, to guarantee the effectiveness of the phytoremediation assessment, greenhouse trials were also included in the Work Plan. Revegetation in greenhouse started from the beginning of October

2018, by using soil samples withdrawn from the PL1 and PL3 biopile sectors (300 kg/each) and last at least until the end of March 2019 (also according to the life cycle of each plant species) to meet the contingency plan of the LIFE BIOREST project. According to the starting season of the trials (autumn), greenhouse pots experiment consists of 4 treatments (represented by *Trifolium pratense*, *Festuca arundinacea*, *T. pratense* + *F. arundinacea*, and non-revegetated control) in 4 biological replicates for 4 thesis for a total of 32 pots of 15 kg each one to guarantee a high scientific/statistical consistency.
The phytoremediation of soil from bio-pile at the demonstration level started after reaching of the satisfying level of pollutant degradation (June 2019). The revegetation area was created within the SIN, according to the project realized in agreement with the Municipality of Fidenza. To guarantee the full phytoremediation treatment, the observation period was up to November 2019. In situ revegetation showed that all selected species were able to grow well in the soil coming from the biopile, while microbiological analyses indicated a further increase in total cultivable bacteria and fungi as compared to the initial contaminated soil.

SUB-ACTION B3.3 - Experimental test replication on other similar polluted soils | Start: 1/10/2018 | End: 30/6/2019. This sub-action aimed to replicate the remediation protocol on representative soil samples from other polluted sites in Spain and France. An essential deliverable propaedeutic to this action (D1.B3 Map and identification of different hydrocarbon polluted sites) has already been carried out according to the planned schedule: the results of this deliverable posed the basis of sub-action B3.3 since it provided a detailed mapping of the European sites where the LIFE BIOREST protocol could be replicated. In the first months of 2018, stakeholders from these sites have been contacted to make agreements on the soil samples and protocols for the method replication. The action was performed, starting from Fall 2018, on French and Spanish polluted sites. Activities were carried out at CSIC for the Spanish soil and in the Michel Chalot's laboratory at Pôle Universitaire du Pays de Montbéliard for the French one. ACTY produced and provided the consortia inoculum to the partners involved in the test replication. The polluted soil was inoculated with the selected microbial consortium; control was set up without the bioaugmentation. Chemical analyses (UCSC), biological monitoring (UCSC, WEL, UNITO) and ecotoxicological analyses (UNITO) were conducted at time 0 (Oct/Nov) and time 60 days (beginning of 2019) and compare to the abiotic control to assess the efficacy of the LIFE remediation approach on other soils.

RESULTS COMPARED WITH PLANNED OUTPUT, TIME SCHEDULE.
The technical problems observed during sub-Action B3.1 had primary effects on the following revegetation activities. In order not to affect the Project schedule, it was proposed not to change the time schedule of the sub-Actions but to modify some activities of the sub-Action B3.2. The additional samples and activities were performed by the project partners who made additional efforts to obtain the expected deliverables. The involvement of UNITO e ACTY in the sub-Action B3.2 helped to achieve the expected results. Greenhouses activities were performed and followed by UCSC.

ACTION C1 – Monitoring and LCA

- **STATUS OF ACTION:** COMPLETED
- **Foreseen start date:** 01/01/2017 **Actual start date:** 01/01/2017
- **Foreseen end date:** 30/06/2019 **Actual (or anticipated) end date:** 30/11/2019*
  **according to the Letter Amendment no. 3*

- **Action leader:** ARPAE
- **Partners involved in the Action:** ITB, UNITO, CSIC

SUB-ACTIONS DESCRIPTION

Action C1 activities were focused on providing the evolution of chemical, microbiological and ecotoxicological parameters during project activities at the micro, meso and in-situ level. Furthermore, sustainability is an important aspect involved in economic, environmental and social issues, and it is commonly assessed by LCA analysis. This Action also includes a preliminary quantitative analysis of energy efficiency for the full-scale bioremediation process. This kind of monitoring is indeed specific to evaluate soil management impact at GHGs emissions level, while LCA allows having a global evaluation of the overall environmental impact of the entire project.

SUB-ACTION C.1.1 - Chemical monitoring of project activities at micro, meso and in-situ level | Start: 1/1/2017 | End: 30/11/2019. Action C1.1 of the LIFE BIOREST project was enclosed the chemical analyses required to monitor one of the most relevant results of the project, namely reducing the concentration of contaminants through bioremediation activities. The monitoring of the variation of contamination in the microcosm and mesocosms samples have contributed to defining the most suitable fungi and bacteria for the degradation of pollutants.

During the mesocosm trial with the LIFE BIOREST soils, the importance of assessing **bioavailability** during a bioremediation approach was highlighted. Indeed, this measurement provides more risk-based information than that provided by total pollutant concentrations only. In this work, the bioavailability was measured as the fraction of PAHs extracted after 20 h with Tenax (ISO/TS 16751), and it was one of the first studies that use this measurement systematically in the field of bioremediation. The single-time point Tenax extraction method has resulted in a reliable and robust way to determine the bioavailability of PAHs in a comprehensive set of samples from bioaugmented LIFE BIOREST soils. Differences often emerged through measurements of bioavailability between samples from uninoculated, control soils, and samples in which the effect of bioavailability-oriented bioremediation was studied by using the LIFE BIOREST consortia. By showing the usefulness of these measurements in the bioremediation field, these results provide additional environmental scenarios for improved risk-based evaluations based on bioavailability of organic pollutants.

CSIC tested the correlation between different amendments (rice husk, biosurfactants) and PAHs extractability. Unfrozen soil sample and amendments were used. Total PAHs concentrations determined after soxtherm extraction, as well as bioavailable concentrations, determined with Tenax extraction, revealed no significant effect of the amendments on pollutant extractability. These methods have been applied to mesocosm soil samples. Bioavailability through the rapid-desorbing fraction and complete desorption kinetics with Tenax in mesocoms samples were determined. This study is very important in determining the magnitude of the different desorbing fractions present in the soil.

Because the hydrophobicities of BTEX and alkanes (as reflected by their, respectively, low and high octanol/water partitioning coefficients or K_{ow}) fall outside the chemical window most suitable for Tenax extractions, their bioavailability was assessed through the standard OECD 304 biodegradability test with ^{14}C-labelled chemicals in all mesocosms at time-zero treatments.

ARPAE has carried out chemical analysis (PAHs and Hydrocarbons C>12) to verify the biopile bioremediation activity. For each sector, an average sample was made, from the excavation of 6 holes, 2.5 meters deep. The surveys were carried out with a mechanical shovel; 3 soil samples were taken, which were subdivided into 12 aliquots. Chemical monitoring of biopile continued until the achievement of the threshold limits.

SUB-ACTION C.1.2 - Microbiological and ecotoxicological monitoring at the in-situ level | Start: 1/1/2017 | End: 30/11/2019. This sub-action was carried out by ARPAE in collaboration with the University of Parma. The following consolidated ecotoxicological tests were applied according to certified ARPAE methods: *Daphnia magna* (acute toxicity test of *Daphnia magna* APAT CNR-IRSA 8020. Man. 29/2003 or OECD GUIDELINE FOR TESTING CHEMICALS n° 202, april 13th, 2004 (*Daphnia* sp.), acute immobilisation test (certificated BPL), *Vibrio fisherii* (acute toxicity test of *Vibrio fischeri* UNI EN ISO 11348-3), *Eisenia fetida* (determination of acute toxicity to *Eisenia fetida*/*Eisenia andrei* ISO 11268-1:2012), *Folsomia candida* (inhibition of reproduction of Collembola (*Folsomia candida*) by soil contaminants ISO 11267:2014), germination tests (Germination and root extension test UNICHIM 1651:2003). Microbiological monitoring was based on microbial count (UCSC and UNITO) and molecular methods as qPCR and meta-barcoding of bacteria and fungi (UNITO, UCSC, WEL). These methods provided additional information about the microbial community, including the unculturable fraction, which may constitute the majority of soil microbial diversity. The quantification of several targeted genes allowed evaluating the capacity of the microbial community to degrade the pollutant present in the soil.

SUB-ACTION C.1.3 - Socio-economic impact and LCA | Start: 1/9/2016 | End: 30/11/2019

1. Socio-economic impact assessment. The socio-economic evaluation aimed at monitoring of impacts generated by the project during its lifecycle and thereafter. The main socio-economic effects of LIFE BIOREST project have been identified as a growing citizens' sensitivity to environmental emergencies – in particular to soil pollution – and the widening of the proposed bioremediation method at national and European level. Several performance indicators have been defined, such as the number of people, companies, public administrations interviewed and involved in public events, technical meetings, educational initiatives and information campaigns.

The first phase of the Socio-economic impact evaluation was carried out by surveys handed out to the target audience identified during public events and communication actions, which were also aimed at promoting project objectives and showing on-going activities and results.
Surveys were focused on gathering useful data to assess elementary environmental awareness, consistency between such awareness and sustainable behaviours and perceived environmental concerns by citizens. Besides, these tools are useful to address and verify the effectiveness of dissemination activities. Data collection mainly concerns the Emilia-Romagna Region (Italy), in particular the city of Fidenza, where the proposed bioremediation treatments took place, and all areas where research activities are carried out by LIFE BIOREST partners (Italy, France, Spain).
Results of these activities were fully described in the deliverable D1.C1.

The second phase of the Socio-economic impact evaluation was focused on defining the main obstacles to increased use of bioremediation. Polluted sites and especially those that have not adopted decontamination solutions yet, represented the target of this study. The analysis could benefit from the Mapping of contaminated sites (Deliverable D1.B3) and focused on the Italian ones for which we have a higher amount of information about the state of remediation, source and type of pollutants.
A database of hydrocarbon polluted sites of regional and national interest was developed starting from 20 registries produced by the Italian regions. The database represents the first national instrument for

the census of all polluted sites in Italy, where they were classified by region, source and type of contaminants with a particular focus on hydrocarbons pollutants and state of implementation of reclamation. Results of these activities were fully described in the deliverable D3.C1.

2. Life Cycle Assessment – LCA: Life Cycle Assessment is a process to evaluate the environmental burdens associated with a product, process, or activity by identifying and quantifying used energy and materials and the waste released to the environment, i.e. it is the systematic analysis of the environmental impact of products during their entire life cycle. Life cycle assessment (LCA) is becoming an increasingly widespread tool in support systems for environmental decision-making regarding the clean-up of contaminated sites. Remediation of a contaminated site reduces a local environmental problem, but at the same time, the remediation activities may cause environmental impacts. LCA is to be used to evaluate the inherent trade-off and to compare remediation scenarios and technologies in terms of their associated environmental burden.
In the LIFE BIOREST project, this evaluation provided technical and scientific evidence to outline the opportunities of bioaugmentation remediation technology, and it allowed to performing a benchmarking of this innovation against other used technologies.
LCA was completed at the end of the project using the data collected during the project. In the first phase of the project, it identified as a clear structure of the new bioaugmentation process. An inventory of the inputs and outputs of the production process and the bioremediation techniques were produced. This inventory provided the basis for the data collected during the on-site biopile comparative remediation activity ongoing in action B3. Beyond the draft of inventory and first "screening LCA" performed to frame the relevant impacts and to understand the main contributions of inputs of the inventory, further data collection was implemented, and the LCA calculation was implemented based on the actual data of the biopile functioning. All the data related to biopile running, VOC emission, on-site energy and water consumption were collected.
During the last reporting period (1/11/2018 -31/12/2019), a complete LCA analysis was performed, and the results were presented during the final LIFE BIOREST event held in PARMA on 18th December 2019 and reported in the final deliverable D4.C1.

RESULTS COMPARED WITH PLANNED OUTPUT, TIME SCHEDULE.
The results obtained by the LCA analysis, conducted by ITB, showed that:

- the bioaugmented biopile displayed better performance than control when considering the amount of HC removed.
- the production of inoculum, specific for the Bioaugmentation biopile, with respect to control biopile, has negligible secondary impacts in all the impact categories taken in consideration in the study, thus does not bring further burdens to the bioremediation process.

Regarding the final risk assessment, conducted by UCSC, all the calculated values resulted lower than the limits of acceptability both for individual and cumulated carcinogenic and non-carcinogenic risks. Therefore it can be said that there are no health risks for visitors caused by the presence of a contaminated aquifer underlying the site.

ACTION D1 – Dissemination initiatives

- **STATUS OF ACTION:** COMPLETED
- **Foreseen start date:** 1/7/2016 **Actual start date:** 1/7/2016
- **Foreseen end date:** 30/6/2019 **Actual (or anticipated) end date:** 31/12/2019*
  **according to the Letter Amendment no. 3*
- **Action leader:** ITB
- **Partners involved in the Action:** UCSC, UNITO, ACTY, ARPAE, CSIC, WEL

SUB-ACTIONS DESCRIPTION

The general communication objectives were focused on informing the broad public about the advantages of the LIFE BIOREST solutions and promote general visibility of the LIFE Programme, encourage the transfer of the project results to other users, other countries and maybe to the European legislative process and spread public awareness about the proper use of natural resources, and pollution prevention.

SUB-ACTION D1.1 Dissemination plan implementation | Start: 1/7/2016 | End: 30/6/2019. The Dissemination Plan was focused on integrated actions aimed at delivering information on innovation in the field of bioremediation, on the return to the city of contamination-free-green-spaces, on the reduction of land use and the dissemination of sustainable practices for the biotech and remediation industry sectors. Dissemination initiatives were carried out according to the timeline and the workplan.

SUB-ACTION D1.2 - Demonstration events, educational visits and public events | Start: 1/7/2016 | End: 30/12/2019. ITB played a crucial role in organizing events aimed at strengthening school involvement. In this regard, additional actions were introduced to involve students in research and communication activities, such as an ideas' competition (**#VerdePerTutti**) created to promote soil and environmental protection (www.lifebiorest.com/ambasciatori-della-scienza).

During the project, **34 public events**, congresses and workshops were organized by the LIFE BIOREST with more than 2.638 attendees involved. A summary table and a detailed description are reported below.

Table 1 *Summary of LIFE BIOREST events according to the main type and number of attendees*

YEAR	TYPE OF EVENT	N OF EVENTS	N ATTENDEES
2016	Kickoff meeting and stakeholder meeting	2	80
2017	Bioremediation lesson for Secondary School students	4	119
2017	Bioremediation lesson for High School students	3	122
2017	Scientific conference, including Annual meeting	5	357
2017	Open day & Networking public seminar	2	241
2017	"LIFE BIOREST ambassadors" training for high school students and communication contest of soil protection	2	400
2017	Green protection day workshop for High school students	1	224
2017	Bioremediation lesson for High School teachers	1	30
2018	"LIFE BIOREST ambassadors" training for high school students and communication contest of soil protection	2	270
2018	Summer School and Scientific workshop	2	180

2019	Policymakers and stakeholders' workshop	6	485
2019	Bioremediation lesson at LIFE BIOREST testing area	4	150
	TOTAL	34	2.658

1. **Kick-off meeting** (Fidenza, September 9, 2016). A public event opened to LIFE BIOREST major stakeholders, such as the regional authorities, professional associations, researchers and to the general public. **50 attendees** were involved.
2. **Stakeholder meeting** (Fidenza, September 21, 2016). The meeting was aimed at ensuring joint programming of the experimental activities of the project. Local authorities involved in the management of the National Interest Site of the Municipality of Fidenza, Emilia-Romagna Region, the Ministry of the Environment and the Protection of the Territory and the Sea, the Local health agency, took part in the meeting. **30 attendees** were involved.
3. **1st seminar for High School students** (Piacenza - UCSC labs, March 16, 2017). The first event dedicated to the protection of the soil, which has inaugurated an environmental education program for **70 students** from the "Lorenzo Respighi" and "Raineri - Marcora" high schools in Piacenza.
4. **Scientific conference** (Milan, March 18, 2017). The meeting organized in collaboration with the Lombardy Green Chemistry Cluster – LGCA, was an opportunity for discussing the role played by biotechnology in remediation applications. **50 attendees** were involved.
5. **Technical lesson** (Lodi, April 19, 2017). A lesson focused on soil pollution, biotech solutions and bioremediation approach. **12 students** were involved.
6. **Seminar for Secondary School students** (Monticelli d'Ongina, May 17, 2017). The event inaugurated the cycle of lessons planned by LIFE BIOREST to involve schools of the Piacenza area. **19 students** were involved (***Fig. 8***).
7. **Seminar for Secondary School students** (Fiorenzuola d'Arda, May 19, 2017). The second lesson focused on environmental emergencies and soil remediation. **36 students** of the "Terre del Magnifico" School were involved (***Fig. 8***).
8. **Seminar for Secondary School students** (Cortemaggiore, May 24, 2017). Third ecology lecture promoted by the Institute of Microbiology of the UCSC. **45 students** were involved.
9. **Seminar for Secondary School students** (Caorso, May 27, 2017). The fourth lesson focused on soil protection and biotechnology solutions adopted by LIFE BIOREST. **19 students** were involved.
10. **"Research and Innovation in the Green Chemistry sector". Bioeconomy stakeholder meeting"** (Milan, May 8, 2017). The conference promoted a dialogue between universities, companies, clusters and public authorities on bioeconomy, environmental policies, research and development issues. The Lombardy Green Chemistry Association attended it, a cluster of 40 research centres and companies operating in the green chemistry field and the Lombardy Region delegates for Research, Innovation, Agriculture and Environment. **143 attendees** were involved.
11. **Seminar for young researchers and students of the Biology and Biotechnology faculties** (Milan – University of Milan, Milan 25, 2017). The seminar was focused at showing the LIFE BIOREST objectives and expected results and on promoting new green jobs in the bioremediation sector. **34 students** were involved.
12. **"LIFE Programme OPEN DAY: Green pathways to the sustainable use of resources. LIFE programme success through its witnesses"** (Milan – University of Milan, May 26, 2017). The Open Day was designed as a contribution to the **25th LIFE Programme Anniversary** (http://life-25.eu/life-programme-open-day-green-pathways-to-sustainable-use-of-resources/), supporting European environmental policies and enhancing results of LIFE-funded research projects for society and the environment. The event provides an overview of the LIFE objectives, strategy, results and success, focusing on disseminating awareness to the public, the scientific community and the industry in the field of environmental protection and responsible use of resources. The LIFE Programme OPEN DAY

was also structured as a "networking" initiative: **6 LIFE projects** (LIFE BIOREST, LIFE DOP, IPNOA, VITISOM, FORAGE4CLIMATE, GAS-OFF) took part in the event and illustrated their main achievements to the public. **83 attendees** were involved.

13. **"Green Jobs for a Greener Future."** (Milan – Research National Council, June 9, 2017). The conference, partner of the EU Green Week (www.eugreenweek.eu) was aimed to engage students, researchers and entrepreneurs interested in dialogue on sustainable and responsible growth issues (http://www.eugreenweek.eu/partner-events/green-jobs-greener-future). The EU Green Week is an annual opportunity to debate and discuss European environmental policy. Organised by the European Commission's Directorate-General for Environment, this key event in the environment policy calendar attracted policymakers, leading environmentalists, stakeholders and other interested parties from across Europe and the globe. LIFE BIOREST took part in the event together with **19 LIFE and non-LIFE projects** active in the environment, green chemistry and agro-food sectors. **158 attendees** were involved.
14. **Seminar of High schools** (Fidenza, September 19, 2017). The event was designed to involve the students of the "Paciolo D'Annunzio" and "IPSSAR S. Solari" high schools of Fidenza in the environmental training programme. **40 students** were involved.
15. **"Ambassadors of Science. Biotechnologies are for you!" An ideas competition for soil protection** (Bologna – Fondazione Golinelli, September 25, 2017) In the context of the European Biotech Week (www.biotechweek.org), LIFE BIOREST organized a public seminar (www.lifebiorest.com/ambasciatori-della-scienza) focused on strengthening awareness about soil protection and bioremediation of contaminated land. "Ambassadors of Science" involved 10 High and Secondary Schools of Bologna and Modena, for a total of nearly 200 students, who were the protagonists of the "bioremediation game", an interactive quiz focused on strategies and biotechnological methods for soil remediation. During the event, **#VerdePerTutti** was launched**,** an idea competition aimed at involving students on pollution issues, particularly soil, its prevention and treatment, and encouraging them to become active in raising public awareness. The initiative is part of an environmental education course open to **10 high schools** in Bologna and Modena (Emilia-Romagna region), that includes laboratory, communication and scientific dissemination experiences related to contaminated soil, bioremediation, pollution prevention and environmental protection (***Fig. 10***). **200 students** were involved.
16. **Green protection Day. Biotechnologies for environmental protection** (Turin – University of Turin, September 29, 2017). In the context of the the European Biotech Week (www.biotechweek.org), LIFE BIOREST organized a public seminar to raise awareness on environmental issues related to soil protection and its bioremediation with biotech solutions (***Fig. 6***). The proposal is linked to the European petition #salvailsuolo, aimed to recognizing soil as a common heritage that needs protection at European level, as it ensures food security, biodiversity conservation and climate changes regulation. The "Green Protection Day" was attended by **224 students** from High Schools which have already collaborated with MUT - Mycotheca Universitatis Taurinensis of the Turin University. The seminar was also opened to sectorial stakeholders, enterprises and public institutions to promote a dialogue on the advantages of industrial biotechnology for health and environmental protection.
17. **Progetto Alternanza Scuola Lavoro with the Piedmont area high schools.** In Italy, the National Project 'Scuola Alternanza Lavoro' is directed to high school students and foresee additional extra-curricular activities student have to take part. The idea is that they may get in contact with industrial and research realities to be aware about the actual development of theoretical studies, to get inspired for their choice of academic studies and to have a first controlled contact with professional jobs. UNITO was involved in the educational program with high school students. Students have to participate to lab activities for a certain period (from 1 to 3 weeks) and are involved into support actions. From 2017, UNITO hosted more than **20 students** coming from two schools: IIS "Gobetti

Marchesini - Casale - Arduino" of Torino and Liceo Scientifico e Classico Statale "G. Peano – S. Pellico" of Cuneo. Students were involved in training activities aimed to discover the importance of microorganisms in soil, the actual risk of polluted soil, the possibility to perform ecotoxicological bioassays to monitor the safety of soil, the capability of fungi to produce molecules as biosurfactants, etc. The program was based on the LIFE BIOREST activities to give them a clear vision of the application of biological approaches and the possible impact of the ecosystem and human society. During the Summer of 2017 students took part on focused activities and ultimately this led to the creation of an **identification Atlas for fungi**. The Atlas was freely distributed to the high schools to facilitate the role of teachers, giving lessons about fungi and their identification.

18. **"LIFE BIOREST Annual meeting"** (Fidenza, October 7, 2017). The conference provided an opportunity for policymakers dialogue and outline the LIFE BIOREST project results. Experts talked about the application of methods for Fidenza and other polluted soils at national and European level. **30 attendees** were involved.
19. **Bioremediation lessons** (Bologna, October 25, 2017). A training course for high schools' teachers focused on bioremediation issue was organized to improve their skills and labs practices. More than **30 teachers** of 10 schools in Emilia-Romagna region took part. ITB, in collaboration with the Golinelli Foundation (host institution of labs) and ACTY, has developed an educational format dedicated to bioaugmentation of bacteria and biosurfactants production. This educational format can be replicated by teachers at their schools' labs.
20. **Soil protection and remediation workshop. Effective communication tools** (Bologna November 24th, 2017). The seminar focused on media and communication tools to encourage public awareness about soil protection, and its remediation was organized. **200 students** were involved.
21. **Bioeconomy Dialogues** (Varese, November 28th, 2017). LIFE BIOREST through its partner ACTY, attended to the public seminar organized by the Lombardy Green Chemistry Cluster focused on sharing the best projects and practices on bioeconomy and circular economy issues. **80 attendees** were involved.
22. **Bioremediation labs practices** (Bologna, February-March 2018). Students participating in the #VerdePerTutti competition carried out practical laboratory activities according to the formats developed by ITB and ACTY, in collaboration with the Golinelli Foundation. The lab activity aimed to monitoring fermentation of microorganisms (yeasts and bacteria). The use of specific sensors for determining the concentration of oxygen or carbon dioxide allowed estimation of the type of metabolism (aerobic/anaerobic) of the microorganism. The microorganisms and fermentation reactions have been studied to understand the bioremediation method applied by LIFE BIOREST at the Fidenza site. **100 students** were involved.
23. **LIFE BIOREST Ambassadors Video Contest Prize** (Bologna May 15th, 2018). Over **170 students** participated in the #VerdePerTutti contest with **42 promotional videos** produced in order to raising public awareness on soil pollution, bioremediation and sustainable use of natural resources.
24. **International Summer school. "Contaminated soil: management and remediation**" (Turin July 10th – 13th, 2018) The International summer training school was organized by ITB, UNITO, WEL, ACTY and CSIC and was dedicated to project promotion and to the training of the young researchers involved, with particular attention to the techniques and methods application on the field with strong involvement of SMEs partners. The International Summer School took place in Torino and was articulated in 4 in-depth training days. Fidenza municipality was involved for the 1-day trip to the contaminated site of Fidenza; during the trip, other sites of interest were visited (in Asti and in Piacenza). The program included 17 speakers. Among the 72 candidates, **30 participants** were selected accordingly to their CV and motivation letters.
25. **Mid-term scientific workshop. "Towards a European strategy for soil protection"** (Turin, July 13th, 2018). A scientific conference aimed at supporting academia and industry collaboration in the bioremediation sector was organized as the Summer School satellite event. Collaboration among

young researchers was encouraged in order to give them the chance to improve their skills, learn from senior scientists and share their work in an international context. More than 150 people and speakers belonging to 6 different National, and International projects attended the event.

26. LIFE BIOREST workshop "Green strategies for the city of the future. Polluted soils bioremediation, soil consumption reduction and sustainable use of resources" (Bologna, 10 May 2019). The event promoted and organized by LIFE BIOREST showed project achievements to the most significant number of stakeholders, and at the same time broadened the reflection to the wider theme of the safeguard and sustainable use of the terrestrial ecosystem inspired by the goals of the United Nations Agenda 2030. A whole day of work where institutions and some local administrations were involved who promoted good practices to make their cities more sustainable. A second technical part of the day was dedicated to the study of some aspects related to soil remediation and conservation, involving among others, ISPRA and the SOS4LIFE project. On this occasion, the first national mapping of SIN and SIR contaminated by hydrocarbons was distributed, a contribution in favour of the establishment of an integrated system of the state of pollution in Italy, after the successful birth of the National System for Environmental Protection (SNPA). More than 130 participants were registered to the event, which was also supported by the Agronomist and Engineering regional associations.
27. Workshop "Approccio multidisciplinare per lo sviluppo di città ecosostenibili: orti urbani, interventi di riqualificazione ed inclusione sociale" (Torino, 24 September 2019). The workshop presented the results obtained in the course of some projects related to the recovery of marginal areas and the implementation of urban gardens. It involved actors belonging to very different contexts (representatives of academia, local environmental agencies) to address the issue of sustainable development and the redevelopment of urban areas with a multidisciplinary and intersectoral approach and was opened to citizens. 150 attendees were involved
28. School visits (3) to the revegetated area within the Fidenza SIN where LIFE BIOREST took place (Fidenza, October- December 2019). Students of high schools already involved in Bioremediation lessons and labs practices participated as visitors to the revegetated area where LIFE BIOREST took place. More than 150 students participated to the agronomic lessons.
29. BorgoFood 2019 (Fidenza, 3-13 October 2019). Annual event organized by the city of Fidenza focused on the issues of sustainability of food production, "slow" and "green Life" mobility. Several events dedicated to eco-sustainability, reuse and recycling were organized, including a session dedicated to the LIFE BIOREST project and the city's commitment to remediation. BorgoFood was also an opportunity to engage citizens to discuss and share the impact of bioremediation for the restoration of new green spaces and their return to the community. More than 80 people attended the project presentation.
30. The strategy to bring green land back to the community. LIFE BIOREST final workshop (Brussels, 26 November 2019). The meeting was aimed at deepening all the significant aspects of the project and at sharing the final results with the European stakeholders. It was an opportunity for policymakers, public institutions, researchers, and industry to come together to exchange knowledge and practices on the implementation in the EU of soil and land-related SDGs, and particular the Land Degradation Neutrality target. During this meeting, LIFE BIOREST first addressed the challenges related to soil and land degradation in the EU and then it explored the opportunities for urgently needed actions. 15 attendees from 6 different EU regions were involved.
31. LIFE BIOREST Final event (Parma, 18 December 2019). The final public event was organized in Parma to present the final results obtained through LIFE BIOREST project. More than 70 participants registered to the event, which was also supported by the Agronomist Association of Bologna province. 80 attendees were involved.

32. **Invited speakers** (Milano, 19 December 2019). The University of Milano invited the group of UNITO to be held two Seminar to the Master students focused on the Bioremediation of Soils. **30 attendees** were involved.

During the last reporting period, the major findings of the project were presented to International Conferences. A full detailed list is reported below.

***Table** 2 Oral presentations and poster presented at international conferences by the LIFE BIOREST partners*

n	INTERNATIONAL CONGRESS PARTICIPATION
1	Lyon, November 21-24, 2017 Fist International Conference on Microbial ecotoxicology - EcoToxicoMic www.ecotoxicomic.fr/fr_fr/ecotoxicomic-2017 POSTER PRESENTATION Enrichment procedures for the isolation of xenobiotic-degrading bacterial and fungal strains: a focus on microbial ecology by UCSC and UNITO. The congress was focused on the ecotoxicological evaluation of polluted sites. It was possible to discussed with many researchers who had to face complex heterogeneous matrices as the site of Fidenza. This information was then very useful during the experimental phases of the Project.
2	Bologna, September 6-9, 2017 9th International Conference on Environmental Engineering and management. Circular economy and sustainability ICEEM 2017 www.iceem.ro ORAL PRESENTATION: "Biological remediation of soil contaminated by hydrocarbons" by UNITO. The topic of the congress were of particular interest for the Project. Among the different discussions and scientific chats, Federica Spina (UNITO) talked with prof. Maria Gavrilescu about a possible joint PhD program and the sharing of students between the two labs and with prof. Simona di Gregorio and some other industrial partners about possible joint researches. Some of them were also involved as experts for the organisation of the International Summer School.
3	Prague, June 25-29, 2017 BioBio 2017 6th International Symposium on Biosorption and Biodegradation/Bioremediation www.biobio.vscht.cz ORAL PRESENTATION "Bioremediation of contaminated land by autochthonous fungi: LIFE BIOREST strategy" by UNITO Federica Spina (UNITO) talked with many colleagues about the exploitation of fungi for different biotechnological uses. The adapted metabolism of the isolated fungi led to a significant interest and the possibility to start new collaboration. For instance, UNITO then started a scientific common research about fungal enzymes with Luisa Barbieri (University of Modena and Reggio Emilia).
4	Microbial Diversity 2017, Drivers of Microbial diversity, Bari, Italy, October 24-26, 2017. UCSC POSTER PRESENTATION: Exploring the xenobiotic-degrading bacterial and fungal consortia during the selective enrichment procedure.
5	PhD Summer school "Organic Matter and Biological Agriculture". Università di Napoli Federico II, Portici, Napoli July 3-6, 2017. Edoardo Puglisi as invited speaker "Exploiting soil biodiversity: a quest for xenobiotic degrading microorganisms"
6	SETAC Europe 27th Annual Meeting, Brussels, Belgium, May 7-11, 2017. POSTER PRESENTATION. R. Posada-Baquero & J.J. Ortega-Calvo. Effect of rhamnolipid biosurfactant in biodegradation of slow-desorption PAHs in contaminated soils
7	6th International Conference on Industrial Biotechnology - IBIC 2018 (Venice, 15-18 April, 2018) www.aidic.it/ibic2018 ORAL PRESENTATION Screening of anionic biosurfactants production among fungi and bacteria by UNITO. The topics were highly targeted on biotechnological application, including soil treatment. Federica Spina (UNITO) discussed about the scale up the process to be applied in the LIFE BIOREST biopile and the possibility to patent the system.

8	11th International Mycological Congress (IMC11) (15-21 July 2018, San Juan, Puerto Rico). POSTER PRESENTATION Bioremediation of contaminated land by autochthonous fungi: Life-Biorest strategy by University of Turin UNITO
9	XXII Convegno Nazionale di Micologia" dell'Associazione Micologica Italiana, (UMI) September 6-8, 2018, Siena, Italy. The talk won the special prize for the best oral presentation. ORAL PRESENTATION Bioremediation of contaminated soil: a strategy based on fungi and bacteria by University of Turin UNITO The oral presentation was awarded as the best presentation at the congress. Federica Spina (UNITO) talked about soil bioremediation with other colleagues who are currently working on HC and DDT in Poland and Check Republic, and on hydrocarbons in an industrial site in Italy. Besides there was number of questions about the possibility to patent the selected microorganisms.
10	XXXVII ANNUAL MEETING OF THE EUROPEAN CULTURE COLLECTIONS' ORGANISATION (Mosca, 13-15 September 2018) ORAL PRESENTATION Bioremediation and ecological restoration of contaminated soils by fungal and bacterial consortia by UNITO Cristina Varese (UNITO) showed the progress of the Project and discussed about the challenges faced for the preservation of the microbial diversity. This was also useful to depict the required efforts and find a common strategy along the pan-European countries.
11	Remtech (Ferrara, 19-21 September 2018) ORAL PRESENTATION Bio Remediation di suolo contaminato da idrocarburi: approccio LIFE-BIORES del sito di Fidenza by University of Turin UNITO Federica Spina (UNITO) had a number of questions about the possibility to patent the selected microorganisms. It was possible to chat with many industries that are working on polluted soils and waters.
12	8th edition of the International Forum on Industrial Biotechnology and Bioeconomy (IFIB). 27-28 September 2018, Torino, Italy. POSTER PRESENTATION: Combining fungi and bacteria for an efficient bioremediation of contaminated soils by University of Turin UNITO The presentations provided a clear idea about the state of the research at the European level. During the be2be Meetings, it was possible for UNITO to directly discuss with many people of the Bio Based Industries Network.
13	Ecomondo 2018, 8-11 November 2018 ORAL PRESENTATION Industrial hydrocarbon polluted soil: bioremediation by bioaugmentation approach. An LCA evaluation by Consorzio Italbiotec, ITB
14	2nd International Conference on Mycology in MENA (ICM-2018) Suez Canal University Conference, October 16–18, 2018 Prof. Cristina Varese (UNITO) was invited for a plenary lecture on Soil and Fungi Remediation, in a series of international conferences on Mycology, Fungi and Fungal Biology of Arab Society for Fungal Conservation. During the conference, prof. Varese have several networking meetings with other researchers including Prof. Ahmed M. Abdel-Azeem (University of Suez Canal, Egypt). They decided to work on an Erasmus Plus Proposal for next year Call.
15	Italian conference on bioremediation, Mantova (Italy), October 17, 2018 Prof. Edoardo Puglisi (UCSC) has been invited as a speaker to the conference.
16	11º Congreso Ibérico y 8º Iberoamericano de Contaminación y Toxicología Ambiental (CICTA), Madrid, 11-13 July 2018. OREAL PRESENTATION Rosa Posada, Jose Luis Garcia, Manuel Cantos, José Julio Ortega-Calvo. Evolution of Bioavailability of PAHs during Bioremediation of polluted soils. Oral (R. Posada).

17	SETAC Europe 28th Annual Meeting, Roma, Italy, 13-17 May 2018. OREAL PRESENTATION R. Posada-Baquero & J.J. Ortega-Calvo. Implementing desorption extraction methods into bioavailability-oriented bioremediation. Oral (R. Posada).
18	1st Global CleanUp Congress 2018, Coimbatore, India, 21-25 October 2018. OREAL PRESENTATION J. J. Ortega-Calvo, R. Posada-Baquero, J. Vila, J.L. García, M. Cantos. How to influence bioavailability for reducing risks from organic pollutants in bioremediation? Oral (J.J. Ortega).
19	38th Annual Meeting of the European Culture Collections' Organisation (ECCO)_Torino (Italy)_12-14 June 2019 POSTER PRESENTATION: Bio-based remediation of polluted soils: LIFE-BIOREST experience by University of Turin UNITO UNITO was mostly focused to merge its experience on the preservation of the fungal strains in the Collection and to deal with a proper benefit sharing. Moreover, during the congress it was possible to talk with Domenico Davolos (INAIL) about a common research based on the genome sequencing on some fungi isolated from LIFE BIOREST. It is also discussed about submitted future project together.
20	First international Biosurfactants conference Stuttgart (Germany)_25-27 September 2019 ORAL PRESENTATION: Fungi as an undiscovered source of biosurfactants by University of Turin UNITO. The congress merged most of the scientific skills around the world about microbial biosurfactants. Bacteria and yeast are the main microbial producers. Federica Spina (UNITO) discussed about the possibility to use filamentous fungi. This aroused many chats and comments; for instance, many groups shared their problems for the fermentation of fungi and the control of process parameters. She had many suggestions about the technical needs for the isolation and purification of the molecules.
21	FEMS 8th Congress of European Microbiologists., Glasgow, Scotland, 7-11 July 2019. POSTER PRESENTATION Isolation and high-throughput screening of xenobiotic degrading bacterial and fungal strains.
22	FEMS 8th Congress of European Microbiologists. Glasgow, Scotland, 7-11 July 2019 POSTER PRESENTATION: Treatment of contaminated soil mesocosms by autochthonous selected bacterial and fungal consortia. UCSC poster
23	Isolation, characterization and application of soil microorganisms useful for the degradation of recalcitrant pollutants and for the stimulation of plant growth. SISS PhD school, Napoli, 4-7 June 2019. Edoardo Puglisi as invited speaker
24	15th European Conference on Fungal Genetics, ECFG15, Rome 17-20 February 2020 POSTER PRESENTATION: Bioremediation of polluted soils – the role of Fungi, Anna Poli, Federica Spina, Valeria Prigione, Alice Romagnolo, Giulia Spini, Ilaria Re, Edoardo Puglisi and Giovanna Cristina Varese.

Details on the main abstracts of the oral presentations and on posters showed during international congresses are reported in detail in the Communication Agenda - Deliverable Report D2.D1 and in Annex 1.D1.

Other education and dissemination activities:

– **Involvement of Under-graduate students.** Few Bachelor and Master students have been involved in the experimental activities of the project, discussing their final Thesis on the topic:

a) Andrea Zanellati (UNITO) entitled "Biorisanamento di un suolo storicamente contaminato: isolamento, caratterizzazione e selezione di ceppi fungini" with Giovanna Cristina Varese as supervisor and, Federica Spina and Anna Poli as co-supervisors;

b) Giuseppe Bentivegna (UNTO) entitled 'Identificazione di funghi con elevate capacità degradative contro gli idrocarburi mediante un innovativo screening miniaturizzato' with Giovanna Cristina Varese as supervisor and Federica Spina as co-supervisor;
c) Silvia Sartori (UNITO) entitled 'Fungi as new source of biosurfactants' with Giovanna Cristina Varese as supervisor and, Pierluigi Quagliotto and Federica Spina as co-supervisors;

– Photographic Competition. UNITO was involved in a project with two professional photographer (Andrea Lorenzon and Silvia Pastore) to take part to an International Photo Competition. The topic of the competition was the Green Sustainability. LIFE BIOREST project and the reality of Fidenza was taken as an example. Several pictures were taken of the most important figures that all move around the SIN were taken, from the Municipality to the technicians working in the field, from the retieìred workers of the old Industries to the partners of LIFE BIOREST project, etc. Everything was merged in a story-telling document and submitted to the Competition Committee. The goal was to achieve new visibility and to sensitize a new public to sensitive.

SUB-ACTION D1.3 - Networking with other project | Start: 1/7/2016 | End: 30/12/2019. A lot of projects and programmes on soil and environment protection are currently ongoing in Italy and Europe. To achieve synergies between LIFE BIOREST and such initiatives, networking activities to present the project were held through participation in events, such as project communication conferences or meetings.
Synergies with the following other LIFE projects were established as follows:

– S.O.S.4LIFE - Save Our Soil for LIFE LIFE15 ENV/IT/000225 www.sos4life.it
– LIFE HelpSoil - Helping enhanced soil functions and adaptation to climate change by sustainable conservation agriculture techniques http://www.lifehelpsoil.eu/
– NewLIFE - Recupero ambientale di un suolo degradato e desertificato mediante una nuova tecnologia di trattamento di ricostituzione del terreno www.lifeplusecosistemi.eu
– BIOREM - Innovative System for the Biochemical Restoration and Monitoring of Degraded Soils www.biorem.ise.cnr.it
– HORTISED - Demonstration of the suitability of dredged remediated sediments for safe and sustainable horticulture production - www.lifehortised.com
– LIFE SUBSED - Sustainable substrates for agriculture from dredged remediated marine sediments: from ports to pots www.lifesubsed.com
– LIFE AGRISED - Use of dredged sediments for creating innovative growing media and technosols for plant nursery and soil rehabilitation www.lifeagrised.com

Networking activities were promoted to encourage best practices exchange among LIFE and non-LIFE projects, such as:

1. Networking event - Bologna, November 28, 2017. LIFE projects event organized in collaboration with S.O.S.4LIFE and LIFE SAM4CP
2. Networking event - Pisa, January 29, 2018. Bioresnova Project organised a workshop about recovery and valorisation of contaminated soils and sediments through innovative biotechnologies and supported by chemical-physical processes to share expertise and good practises.
3. Mid-term scientific conference. Engagement of 5 other national and European projects, such as: PhytoSUDOE (funded by Interreg Sodoe), S.O.S.4LIFE (funded by LIFE Programme), Kill Spill (funded by FP7), BioresNova and Re-Horti.
4. Networking Event – Torino, September 26, 2018. Pilots4U Horizon Scan Workshop: Equipping Europe for Bio-based Innovation as a satellite event to the International Forum on Industrial

Biotechnology and Bioeconomy (IFIB2018). The Pilots4U project has set up an easily accessible database of open access pilot and demonstration infrastructure for the European bioeconomy.

ACTION D2 – Dissemination and communication materials

- **STATUS OF ACTION:** COMPLETED
- Foreseen start date: 01/07/2016
- Foreseen end date: 30/06/2019

Actual start date: 01/07/2016
Actual (or anticipated) end date: 30/12/2019*
**according to the Letter Amendment no. 3*

- **Action leader:** ITB
- **Partners involved in the Action:** UCSC, ARPAE

SUB-ACTIONS DESCRIPTION

The planning and development of promotional materials and digital communication was carried out by ITB. Material features and management methods was detailed in the Dissemination Plan (DP).

SUB-ACTION D2.1 - BIOREST LIFE logo | Start: 1/7/2016 | End: 30/06/2019. ITB (CB), as the manager for communication activities, developed the project logo and brand usage guideline useful for PowerPoint presentations, public presentations, press releases to define a coordinated image of project identity. ITB guarantees the correct use of the **LIFE logo** on documents, visual supports and durable goods, giving instructions on this to all partners during meetings, seminars and workshops.

The Brand usage guidelines and obligations about the LIFE logo were reported in the **Dissemination Plan** (M1.D1) and **Project Management Handbook** (ANNEX 1.E1)

SUB-ACTION D2.2 - Project leaflet and notice board | Start: 1/7/2016 | End: 30/6/2019. ITB developed a project newsletter, in order to disseminate information about the project (objectives, intermediate results, events), demonstration events and any information related to the topics covered by the project of national or global interest). All newsletter issues produced are freely downloadable from the website and sent to registered users. The newsletter has a corporate identity with the site of the project. It is possible to sign up to the project newsletter via the project website (a specific banner is available on all pages). Social network campaigns to promote signing up to the newsletter were also designed. The first issue of the project newsletter was sent to over 2,500 subscribers in **May 2017**.

- Leaflet. Leaflets include a general overview of the project, main objectives, project context, LIFE and partner logos, goals and members, highlighting the importance of the results and main areas of interest for potential users.
- Notice board. Poster diplayed at project partners' premises and at Fidenza testing site aimed at communicated at non-technical languageobjevtive and impact of the project.
- "Soil Bioremediation" leaflet. It illustrates facts and figures related to soil pollution and its reclamation through biological technologies. The guide has been distributed during the School and the international conference (Turin 13th July), it is also published in a digital version on the project website and promoted on social networks (Facebook project page) – attached in this report.
- State of hydrocarbon pollution in Italy infographic. The leaflet summarized fact and figure of the state of contamination as reported in the project publication "Soil contamination from hydrocarbons in Italy. A mapping of progress in reclamation of Site of National and Regional interest".
- Banner and backdrop. Produced to display the mission of the project during public events.

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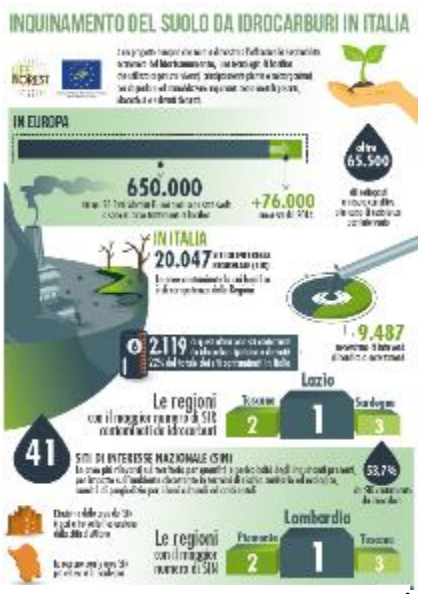

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1. Leaflet (A4 format double sider) distributed in digital and paper version; **2.** Notice board (poster format 50x70 cm) displayed at project partners and Fidenza testing area sites. **3**. Soil bioremediation leaflet (A5 format) distributed in digital and paper version. **4**. State of hydrocarbon pollution in Italy (A4 format) distributed in digital and paper version; **5**. Banner (3x2 m) and backdrop (6x3 m)

- Gadgets and Promotional banner. In order to increase the public engagement in the LIFE BIOREST project, through visits to the website and newsletter signup, LIFE BIOREST merchandising and graphic materials were developed, such as **pens**, **pen-drives**, **wristbands, roll-up** and **block notes.**

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6. LIFE BIOREST gadget (pens, pen-drivers, wristbands, block notes, distributed during public events and workshops; **7.** Roll-ups (200x80 cm); **8.** "Soil contamination from hydrocarbons in Italy (Italian and English versions), After-Life Plan and Layman's report; **9.** Environmental Guidelines (English, French, Spanish versions)

- "Soil contamination from hydrocarbons in Italy". A mapping of the state of hydrocarbon contamination in Italy (Italian an English version). The second part of the socio-economic Study is focused on mapping the state of pollution and the main obstacles to the full application of the bioremediation method. A database of all the sites of regional and national interest has been developed starting from 20 registries produced by the Italian regions.
- "**Environmental Guideline for contaminated soil bioremediation**". It represents the final publication of LIFE BIOREST project produced in three languages – English, Spanish and French, representing a useful tool for the public authorities and decision makers who is facing the problem of managing a hydrocarbon polluted site all over Europe. It aims to demonstrate benefits of bioremediation among local authorities where soil pollution is unsolved problem.

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SUB-ACTION D2.3 - Press campaign, publication in Scientific Journals and Project manual | Start: 1/7/2016 | End: 30/12/2019. During the project **15 press releases** were published by LIFE BIOREST and other online newspapers, blogs and project stakeholders' media. The production of this material aims to promote events, seminars and conferences organized by LIFE BIOREST among magazines and local newspapers. From the project beginning **1 newsletter**, **3 articles** on the leading technical national magazines and **6 scientific articles** with the highest uptake in the bioremediation sector were published. Finally, **31 newspaper articles** on local daily newspapers were published. All of the articles are available on the project website under the "Publications" section.

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All of the articles are available on the project website under the "Publications" section.

Press releases published during LIFE BIOREST public events

	AUTORS	EVENT AND PRESS RELEASE PRODUCTED
1.	CONSORZIO ITALBIOTEC	LIFE BIOREST Public Kick-off Meeting. September 9, 2016
2.	COMUNE DI FIDENZA	LIFE BIOREST Public Kick-off Meeting. September 9, 2016
3.	LIFE BIOREST	Annual Meeting. Fidenza October 7, 2017
4.	COMUNICATI-STAMPA.NET	Green Protection Day. Green biotechnologies for environment protection. Torino, September 29, 2017
5.	LOMBARDIAPRESS	Green Protection day. Green biotechnologies for environment protection. Torino, September 29, 2017
6.	LIFE BIOREST	@EUBiotechWeek: Green Protection Day. University of Turin opens doors to 250 students to talk about soil, pollution and environment protection
7.	LIFE BIOREST	@EUBiotechWeek: Science Ambassadors. Biotechnologies are for you! 200 students welcomed at Golinelli Foundation. Start idea competition #VerdePerTutti (Green for all)
8.	LOMBARDIAPRESS	EU Biotech Week Bologna, September 25, 2017
9.	LIFE BIOREST	Biological remediation of contaminated site: the European project LIFE BIOREST meets 50 students. Fidenza, 19th September 2017
10.	LIFE BIOREST	LIFE Programme Open Day. May 26, 2017

11.	LOMBARDIAPRESS	Lombardy Green Chemistry Cluster. March 30, 2017
12.	LIFE BIOREST	"Towards a European strategy for soil protection", Turin July 13th, 2018
13.	LIFE BIOREST	"Top of world microbiology tells the model of Fidenza reclamation". In collaboration with Fidenza Municipality
14.	LIFE BIOREST	LIFE BIOREST workshop "Green strategies for the city of the future. Polluted soils bioremediation, soil consumption reduction and sustainable use of resources" (Bologna, 10 May 2019).
15.	LIFE BIOREST	LIFE BIOREST Final event (Parma, 18 December 2019).

Articles published on the main technical national magazines

	NAME OF MAGAZINE	TITLE OF ARTICLE
1.	RECOVER MAGAZINE, N°37 2016	I. Re, *Bioremedtion, revegetation and ecological restoration at Ex Carbochimica of Fidenza*, pp. 46-49
2.	eCOSCIENZA, N° 4 2017	E. Puglisi, G. Spini, F. Spina, G.C. Varese, *Bioremediation of polluted soils contaminated by hydrocarbons*, pp. 16-18
3.	Platinum	*Green strategies for cities of the future*, April 2019
4.	Arbor n 3, 2018	Re I., Rigenerazione urbana: modelli sostenibili per la bonifica del suolo. LIFE BIOREST, un progetto europeo per il biorisanamento

Scientific articles published on the scientific journals.

	NAME OF SCIENTIFIC JOURNAL	TITLE OF ARTICLE
1.	CHEMICAL ENGINEERING TRANSACTIONS	Federica Spina, Giulia Spini, Anna Poli, Alice Romagnolo, Andrea Zanellati, Nicolò G. Bentivegna, Najoi El-Azhar, Tiffanie Regnier, Anne-Laure Blieux, Abdelwahad Echairi, Valeria Prigione, Edoardo Puglisi, Giovanna C. Varese. *Screening of Anionic Biosurfactants Production among Fungi and Bacteria*
2.	FRONTIERS IN MICROBIOLOGY	Giulia Spini, Federica Spina, Anna Poli, Anne L. Blieux, Tiffanie Regnier, Carla Gramellini, Giovanna C. Varese, Edoardo Puglisi. *Molecular and microbiological insights on the enrichment procedures for the isolation of petroleum degrading bacteria and fungi*
3.	ENVIRONMENTAL SCIENCE & TECHNOLOGY	Rungroch Sungthong, Margalida Tauler, Magdalena Grifoll and Jose Julio Ortega-Calvo, *Mycelium-Enhanced Bacterial Degradation of Organic Pollutants under Bioavailability Restrictions*
4.	SPRINGER INTERNATIONAL AG 2017	Julio Ortega-Calvo, *Strategies to increase Bioavailability and Uptake of Hydrocarbons*
5.	ENVIRONMENTAL ENGINEERING AND	*Giuliana D'Imporzano, Ilaria Re, Federica Spina, Cristina Varese, Edoardo Puglisi, Giulia Spini, Carla Gramellini, Giacomo Zaccanti, Fabrizio Beltrametti, Adriana Bava, Fabrizio Adani*

	MANAGEMENT JOURNAL	*Optimizing bioremediation of hydrocarbon polluted soil by life cycle assessment (lca) approach*
6.	SCIENCE OF THE TOTAL ENVIRONMENT	*Rosa Posada-Baquero, Maria Lopez Martin, Jose Julio Ortega-Calvo, Implementing standardized desorption extraction into bioavalability-oriented bioremediation of PAH-pollutes soil*

During the meeting among Partners of March 3rd 2020, the schedule of the upcoming papers has been discussed. With a complete overview of the results, Partners agreed to work together to have few papers submitted in the upcoming months as described below:

NAME OF SCIENTIFIC JOURNAL	TOPIC / RESULTS	PARTNER responsible of writing	PARTNERS involved in the publication
1. FRONTIERS IN MICROBIOLOGY	Microbial isolation and characterization (Action B1.1)	UNITO	UNITO, UCSC, WEL, ACTY
2. To be defined	Microcosms and mesocosms (Action B1.3)	UCSC	UCSC, UNITO, ACTY, ARPAE
3. BIORESOURCE TECHNOLOGY	In situ biopile and revegetation (Action B3.1-3.2)	ACTY	ALL
4. To be defined	Greenhouses Trials (Action B3.2)	UCSC	UCSC, UNITO, ACTY, ARPAE
5. To be defined	Testing activities (Action B3.3)	UNITO	UNITO, UCSC, WEL, ACTY, CSIC
6. To be defined	LCA on in situ trials (Action B3)	ITALBIOTEC	ALL
7. To be defined	Biosurfactants (Action B1)	UNITO	UNITO, UCSC, ACTY

Newspaper articles published on daily newspapers

	ISSUE	NEWSPAPER
1.	September 9, 2016 – life biorest kick-off meeting	Gazzetta di parma
2.	September 9, 2016 – life biorest kick-off meeting	Il parmense
3.	September 9, 2016 – life biorest kick-off meeting	Pamadaily
4.	September 9, 2016 – life biorest kick-off meeting	Gazzetta di parma
5.	September 9, 2016 – life biorest kick-off meeting	Piacenza sera
6.	December 21, 2016 – live streaming	Telelibertà
7.	November 4, 2016 – biorest among soil protection eu project	Europanatura
8.	September 29, 2017 – torino, green protection day	Obiettivonews
9.	September 19, 2017 – fidenza seminar for high schools	Parmapress24
10.	September 19, 2017 – fidenza seminar for high schools	Parmabilly
11.	September 19, 2017 – fidenza seminar for high schools	Parma repubblica
12.	June 9, 2017 – biorest partner of #eugreenweek	Green planner magazine
13.	June 26, 2017 – life programme open day - #25lifenatura	Green planner magazine
14.	May 17, 2017 – fungi and bacteria for bioremetiation	Libertà piacenza
15.	March 16, 2017 – fist seminars for high schools	Libertà piacenza
16.	March 16, 2017 – fist seminars for high schools	Il piacenza

17.	Ex cip e carbochimica di fidenza: le bonifiche proseguono	Parma repubblica
18.	I funghi mangia inquinamento: bonificheranno i terreni dell'ex carbochimica	Greenme
19.	La fabbrica dei funghi che puliscono il terreno dall'inquinamento	La stampa
20.	Life biorest: ecco i funghi mangia inquinamento	Io penso positivo
21.	Saranno i funghi a liberarci dalle sostanze inquinanti?	La stampa
22.	On air interview to prof. Cristina varese	Radio rai
23.	June 2018 - Batteri e funghi riqualificano i suoli	Green planner (2018)
24.	June 2019 - Il biorisanamento funziona	Green planner (2019)
25.	Il modello "green" sotto i riflettori internazionali	Gazzetta di parma
26.	Ecco come i funghi ci salveranno dall'inquinamento	Ehabitat
27.	Bonifica siti contaminati: progetto europeo arruola 50 studenti di fidenza	Parmapress
28.	Bonifica siti contaminati: progetto europeo arruola 50 studenti di fidenza	Parmabilly
29.	April 2019 – Life Biorest: come ripulire i terreni contaminati da petrolio con funghi e batteri	Il Piacenza
30.	May 2019 - Strategie Green per la città del futuro	La Stampa
31.	June 2019 – I funghi: futuri protagonisti delle bonifiche ambientali	Pimonte che cambia

SUB-ACTION D2.4 - Layman's report | Start: 1/7/2016 | End: 30/12/2019. The Layman's report has been elaborated and completed during the last months of the project, reporting a summary of the project objectives, the actions and related results.

SUB-ACTION D2.5 - Project website and newsletter | Start: 1/7/2016 | End: 30/12/2019. The LIFE BIOREST website was expected to attract individual visitors as well as stakeholders with interest in bioremediation and ecology issues and constitute an essential source of information for public authorities, relevant to their decision making. Academic and technical audiences also have the opportunity to benefit from the published reports and research data. The dedicated "Documentation and Press" section allows readers to download all project dissemination documentation and practical information for expert and non-expert audiences. Besides, journalists can find information sources in the "Press review", "Press releases" and "Blog" sections. The LIFE BIOREST website meant to fulfil the following functions:

- To serve as a dissemination channel for the various communication materials that will be produced along with the project
- To provide material for press & specialised media professionals and to collect the reports of the project on these media
- To provide information about events related to decontamination of polluted soil, especially those events in which LIFE BIOREST participates

To serve as a connection channel to social media networks such as Twitter, LinkedIn etc

The project website was set up under the URL www.lifebiorest.com. As Coordinating Beneficiary (CB), ITALBIOTEC (ITB) was responsible for the website hosting, design, correct functioning and contents update. The domain will be kept registered for at least 5 years beyond the project's end date.

The website of the project was developed in both Italian and English, in consideration that this is not only the official language of the project but also the primary communication language if the technology must reach a broad audience. The LIFE BIOREST project website was implemented and updated during

the project, according to the project progress timeline. It was launched at the end of October 2016.

Figure 15 *- The Life BIOREST website homepage*

Leading project **website performance indicators** related during the last reporting period (from 1.11.2018 to 31.12.2019), are reported below.

- Total number of users reached: **2.851, of which 2.841 new users**
- Total number of sessions: **3.601** (1,26 session per user)
- Total number of visualisations per page: **7.656**
- New visitors: **89,6%**
- Returning visitors: **10,4%**

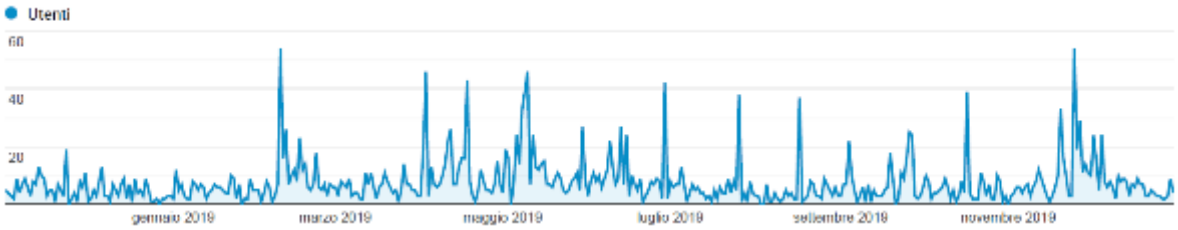

Figure 16 *- Monthly trend of users reached during the reporting period (2.851 users). Website performance indicators are registered by Google Analytics.*

During the overall project duration (from 1.7.2016 to 31.12.2019) are reported below:

- Total number of users reached: **7.327**
- Total number of sessions: **10.227** (1,40 session per user)
- Total number of visualisations per page: **24.012**
- New visitors: **86%**
- Returning visitors: **14%**

Figure 17 *- Monthly trend of users reached during the project life from 1.7.2016 to 31.12.2019 (7.327 users).*

SUB-ACTION D2.6 Project videos | Start: 1/7/2016 | End: 30/12/2019. During the LIFE BIOREST, project

videos focused on the dissemination and promotion of project objectives and results are being developed. These are live streaming videos filmed during the seminars and demonstration events, but also interview, video-animations, spots. Videos include the LIFE logo and a clear reference to the LIFE financial support. Videos are available on the project website and disseminated through social network campaigns on LinkedIn, Facebook and YouTube. During the project lifecycle, **55 videos** were developed to disseminate project goals and results and increase knowledge about soil pollution. Concerning the primary target audience of LIFE BIOREST (general public, students, researchers, entrepreneurs and policymakers), different content and messages were identified. Three respective types of videos have been designed, labelled within the project website as "Educational", "Interviews" and "Events." (https://www.lifebiorest.com/documenti)

All videos are also available within the YouTube channel of the Consorzio Italbiotec (CB), collected within the Playlist called "LIFE BIOREST, a strategy for soil protection".

More videos than expected were also produced, to increase visibility and public participation in project activities. Thanks to the LIFE BIOREST Ambassadors initiative (Bologna May 15th, 2018) over 170 students participated in the #VerdePerTutti Video Contest with **42 promotional videos** produced to raising public awareness on soil pollution, bioremediation and sustainable use of natural resources. All videos are also available within the YouTube channel of the Consorzio Italbiotec (CB), collected within the Playlist called "LIFE BIOREST, a strategy for soil protection".

In collaboration with Striscia la Notizia (Mediaset network, Italy), a video was produced dealing with the approach and the major findings of LIFE BIOREST. The video was taken in the UNITO lab in December 2018, also interviewing prof. Cristina Varese. The video was nationally released in Spring 2019.

SUB-ACTION D2.7 Social network promotion | Start: 1/7/2016 | End: 30/12/2019. The LIFE BIOREST project has a presence on selected social network communities (Facebook, Twitter, LinkedIn), which play an essential role in the overall communication and dissemination work. The profiles are integrated on the website as feeds, thus functioning as an effective way of keeping the website updated. Content and inbound marketing were designed to increase visits to the project website, the number of newsletter subscribers and video views.

Since the beginning of the project, **18 promotion campaigns** on Facebook Ads and Google Awards were launched. The main objectives of advertising ads are: (1) Increasing event participation, (2) Connecting people to the LIFE BIOREST fan pages, (3) Increasing video views, (4) Promoting project outcome. Each produced ad should refer or link to a form for newsletter subscription, thus ensuring a repeatedly and flexible manner of communicating to project stakeholders.

The main results are summarized below:

Total number of LIFE BIOREST Facebook follower	**2.000**
Total number of people reached / contents displayed (via Facebook)	**191.619**
Total of “like” of LIFE BIOREST Facebook fan page	**1.980**
Number of posts published	**+ 50**

ACTION E1 – Project Management

- **STATUS OF ACTION:** COMPLETED
- Foreseen start date: 01/07/2016 — **Actual start date:** 01/07/2016
- Foreseen end date: 30/06/2019 — **Actual (or anticipated) end date:** 31/12/2019*
  **according to the Letter Amendment no. 3*
- **Action leader:** ITB
- **Partners involved in the Action:** UNITO, UCSC, ACTY, ARPAE, CSIC, SATT SAYENS

SUB-ACTIONS DESCRIPTION

Monitoring activities were carried out by the ITB and consisted of evaluating the efficacy of planned actions and verifying that the project's objectives were achieved. To prepare comprehensive and compliant financial reports, the LIFE BIOREST Consortium established a reliable system for collecting administrative and financial data of each AB. ITB organised a private folder on Dropbox Platform, where ABs were invited to upload timesheets, invoices, contracts, financial statements and any other useful file, for LIFE reporting purposes. Every quarter the CB checked all the files uploaded, registered the information, requested clarifications where needed. The private folder was useful also to share Guidelines, Templates, Minutes, Monthly report, link to LIFE Toolkit on the Internet as well as internal Minutes, Letters from the European Commission. The CB was able to monitor costs and to compare them to the budget continuously.

SUB-ACTION E1.1 - Project management by ITB | Start: 1/7/2016 | End: 31/12/2019. The Project Coordinator was responsible for the full implementation of the project and maintenance the contacts with the Commission and stakeholders to achieve the project activities and to disseminate the project results. According to the Grant Agreement, each ABs were directly involved in the technical implementation of one or more tasks of the project. The Management Staff, with representatives of all partners, had a transversal role with decision power and oversaw project implementation. At each meeting or videoconference, were documented by the Minutes. The work plan set down was regularly updated following the general scheme of the Gantt chart. Moreover, the Gantt Chart was discussed and shared at each meeting, for reassurance about the consensus.
Main outcomes of Sub-Action E1.1. are:

- **Project Management Handbook** focusing on administrative and financial reporting was distributed on July 25, 2016, to check the compliance of the AB to the Common Provisions rules. This document has been updated on a private folder and discussed during the Public Kick-off Meeting on September 9, 2016 (ANNEX 1.E1).
- **Contingency Plan** to evaluate potential mitigation and response actions to reduce potential delays in project achievements and provide solution and protocol optimizations. Project management staff and Action leaders met regularly to discuss and develop actions while providing oversight of data analysis, reviewing work productions and participated in public outreach efforts. The Contingency Plan serves as a source of information and planning consensus to manage main deviations from the work plan (ANNEX 2.E1).
- **Partnership Agreement** was signed on March 13, 2017. It defines the organisation between partners, the management of LIFE BIOREST, rights, obligations, IP and ethical rules within the project. The purpose of this agreement is to achieve progressive complementarities and integration among the partners and to establish sustained structures. An amendment to the **Partnership Agreement** was signed between ITB and ARPAE on **August 2019** to formalize the budget relocation for the design and development "Environmental Guideline for contaminated soil bioremediation" a task relocated to ITB (ANNEX 3.E1).
- **Financial and Administrative periodic evaluations**. Monitoring activities were carried out by the CB

and consisted of evaluating the efficacy of planned actions and verifying that the project's objectives had been achieved. In order to prepare comprehensive and compliant financial reports, the LIFE BIOREST Consortium established a reliable system for collecting administrative and financial data of each AB. CB created a Dropbox folder as a private area, where ABs are invited to upload timesheets, invoices, contracts, financial statements and any other useful file, for Life reporting purposes. Every quarter the CB checked all the files uploaded, registered the information, requested clarifications where needed. A second Dropbox folder open to all Project Partner was created in order to share Guidelines, templates, internal minutes, letters from the European Commission, deliverables and milestone. The CB was able to monitor costs and to compare them to the budget on a continuous basis.

- Monitoring meetings were organized in order to discuss and solve any technical or administrative problem eventually encountered. During the project lifetime, 43 meetings were organized (including video-conferences),

6.2 Evaluation of Project Implementation

The strategy of the LIFE BIOREST project is based on three key points: Identify, Produce, Apply.

- **Identify.** Identification of the pollutants as well as of the actors (microorganisms, stakeholders, communities) involved in the bioremediation process is crucial.
- **Produce.** The LIFE BIOREST approach produces the following: **microorganisms** for the application the area to be remediated; **awareness** of the population about the advantages of having bioremediation over another approach and investment on the future of the area; **control** by use of ecotoxicological data to increase the awareness and the acceptance not only of the method applied but also on the possibility to have back land for several purposes (recreational, residential).
- **Apply.** The application of microorganisms, biostimulants, and awareness is of fundamental importance for the whole remediation process itself.

ACTION	FORESEEN IN THE REVISED PROPOSAL	ACHIEVED	EVALUATION
Action B1 – Optimization of soil bioremediation protocol	• **Objective.** Selection of the strains for the further exploited in the treatment of contaminated soil. • **Result.** Optimize protocol for bioremediation potentially applied in other scenarios	Optimized and tailored protocol based on a combination of microbial consortia with improved degradation abilities towards alkanes, PAHs, BTEX	Soil treatment was optimized by the isolation of 350 fungi and bacteria selected according to their degradation abilities and tested at micro and mesocosm levels.
Action B2 – Upscaled production of microorganisms	• **Objective.** Demonstrate the efficiency and cost-effectiveness of a bioremediation approach • **Results.** Large-scale production of microorganisms in situ activities	Validated scale-up method to produce microorganisms able to guarantee efficient bioremediation and demonstration area of 400 m^3 in the site of "ex-Carbochimica."	Cost-efficiency and cost-effectiveness of microorganism production were proven. The cost of bioremediation could range approximately from € 200-1,000 per ton of soil.
Action B3 – In situ bioremediation and revegetation	• **Objective**. Revegetate the decontaminated soil, restore its ecological function • **Result.** In situ bioremediation and revegetation of 400 m^3 polluted soil .	Feasibility study for LIFE BIOREST wide range testing was supported by in situ bioremediation and revegetation of an area of 400 m^3 inside of the "ex-Carbochimica" site.	Bioremediation and revegetation of polluted soil were achieved. Experimental tests replication on other polluted soil in Spain and France were successfully proved. A fungal treatment of a polluted urban garden was run by UNITO, thanks to the methodology developed by LIFE-BIOREST.

Action C1 – Monitoring and LCA	• **Objective.** Monitoring the efficiency of the bioremediation methods • **Results.** Chemical, microbiological, ecotoxicological monitoring	Chemical, microbiological, ecotoxicological monitoring, socio-economic impact, and Life Cycle Assessment	Demonstration of the bioremediation with bioaugmented and biostimulated microorganisms is cheap and environmentally sound.
Action D1 – Dissemination initiatives	• **Objective.** Spread knowledge and consciousness about environmental pollution • **Results.** Increase knowledge of risk assessment, biodiversity, ecotoxicology, soil ecology, and bioeconomy.	Exploit biological methods for soil bioremediation based among public authorities, the scientific community, industrial sector and general public	Disseminate the clear societal benefits of addressing the issue of soil contamination at the European level
Action D2 - Dissemination and communication materials	• **Objective.** Development of promotional material and digital communication tools • **Results.** Communication pack (logo, factsheet, notice board, website), videos and other digital tools	Communication materials expected by the project fully designed, developed and successfully spreading (communication pack, gadgets and publications)	The LIFE BIOREST primary publication the "Soil contamination from hydrocarbons in Italy" and the "Environmental Guideline for contaminated soil bioremediation" were translate in several languages and successfully disseminated among the project target audience
Action E1 – Project management	• **Objective.** Guarantee the effective development of project activities • **Results.** Reach expected goals and results	Monitoring activities were carried out by the ITB and consisted of evaluating the efficacy of planned actions and verifying that the project's objectives were achieved	LIFE BIOREST Consortium established a reliable system for collecting administrative and financial data of each AB as well as periodic technical reporting

The **lesson learned** was reported in Layman's report. It is targeted at a non-specialist audience and results in an informative tool for decision-makers and non-technical parties of the project objectives and results. It represents, along with the project website, one of the main tools for disseminating information after the project's end.
The report includes an overview of the soil pollution problem and its consequences on the ecosystem, human and animal's health, offering the alternative of the bioremediation based on the approach developed by LIFE BIOREST project, to restore polluted soil affected by hydrocarbon's contamination.

Objectives and actions of the project are reported, along with the benefits of the innovative solution proposed by LIFE BIOREST. The results of the project are described in a non-technical way to reach a wider audience, including non-technical parties.

The Layman's report also intends to highlight the potential of replicability of the approach developed in the LIFE BIOREST project. Indeed, considering the potential of microorganisms, the same strategy could be adopted in improving soil fertility, in speeding up the shifting of soils from traditional to organic agriculture, in preventing (by ready-to-use protocols) pollution by "at-risk" industrial activities. Therefore, LIFE BIOREST has the potential to integrate into several of the different policies of the EU.
In conclusion, from the social point of view, contaminated soil represents a lost economic opportunity and a threat to the health and wellness of human beings and the environment.

Effectiveness of the dissemination activities

The impact of dissemination initiatives is summarized in Key performance indicators as follow:

- **32 public events, seminars, workshops** organized by engaging more than 2,600 people
- **24 oral presentations and poster exhibition** at international scientific conferences
- **1.100 secondary and high school students** involved in bioremediation lessons, seminars and labs
- **30 international researchers** involved in the Summer School
- **30 high school teachers** engaged in bioremediation training
- **2 open day and networking initiatives** organized engaging more than 240 people
- **7 LIFE projects engaged** in dissemination initiatives
- **250 students engaged in a Video-contest for communicating pollution issues**, particularly soil, its prevention and treatment, and encouraging them to become active in raising public awareness
- **55 videos produced**, reaching more than 50.000+ people
- **2.000** LIFE BIOREST Facebook follower
- **191.619** people reached / contents displayed (via Facebook)
- **31 newspaper articles** on local daily newspapers published
- **15 press releases** and **1 newsletter** published by the LIFE BIOREST
- **4 articles** on the leading technical national magazines
- **6 scientific papers on peer-reviewed journals + 7 under preparation (expected by 2020)**

The After-LIFE Plan elaborated within LIFE BIOREST project is intended to give a clear demonstration of the commitment of each partner to contribute to the continuation of the activities during the next 5 years after the end of the project (2020 – 2024) (***Figure 7***).

Each action of the LIFE BIOREST project foresees its continuation through the partners' activities:

- ACTION B1. All the selected fungi are included in the Mycotheca of the University of Turin Database, enlarging the stored biodiversity that could be useful for future biotechnological exploitation. A continuous study of the fungal genome will carry out, thanks to a collaboration with INAIL. Through which the genome of some fungi has been sequenced, and their metabolic and molecular peculiarities are under study. A continuous investigation of secondary microbial metabolites (fungal lipases, bacterial biostimulants) and microbial biosurfactant production, trying to reduce the production costs; the selected microorganisms will be tested on different matrices (e.g. wastewater, industrial water) other than soil, to adapt the developed bioremediation protocol.

- ACTION B2: the optimization of low-cost culture media for bioaugmentation and biostimulation of microorganisms will be carried out, along with the implementation of a protocol for rapid and effective intervention on polluted soils. The developed know-how will value for the development of new bioremediation projects worldwide
- ACTION B3: the identification of plant species that can tolerate highly polluted environments to be tested in other sites of interest will be explored; a complete integrated approach that combines culturomic techniques and next-generation sequencing analysis will be applied to evaluate microbial community of any matrices. The Municipality of Fidenza will be supported in urban development plans to promote the restoration of urban areas.
- ACTION C1: Inventory data of LCA analysis of LIFE BIOREST project will be shared, to disseminate the importance of the use of LCA as an ex-ante and ex-post evaluation tool and to increase the available data on LCA for soil bioremediation

- ACTIONS D1, D2: The following initiatives will support the dissemination and exploitation of project results:
- Project Website maintained for at least 5 years and 1000 views/year.
- Facebook project page updated for at least 5 years and at least 1000 views/year.
- Environmental education for high school students through testing area lessons (1 visit/year)
- Videos on YouTube channel maintained for at least 5 years and at least 1000 views/years
- Communication materials distributed through pendrives during conferences / events /exhibitions. (At least 30 pendrives distributed in 2 events/year)
- Networking activities with similar projects and actors for the progression of theoretical and applied knowledge in the field of soil bioremediation.
- 5 papers published on peer-reviewed scientific journals.
- The After-LIFE communication strategy will be focused on the awareness increasing through the dissemination of two publications elaborated during the project: **"Soil Contamination from Hydrocarbons. Mapping of the progress of Sites of National and Regional Interest**" and "**Environmental Guideline for contaminated soil bioremediation**" where no remediation procedure has been initiated, to reach at least 20 sites. The knowledge arose and the experience gained from the LIFE BIOREST project will be exploited in the development of further projects on soil protection and the use of bioremediation as a promising alternative to conventional methods.

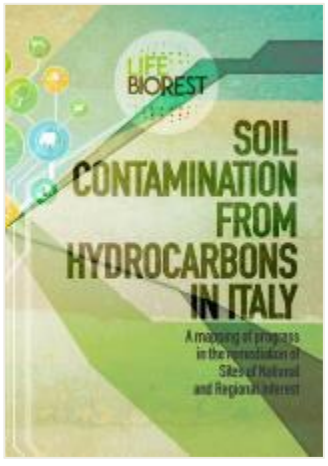

Figure 18 *The LIFE BIOREST publication (from left): "Soil contamination from hydrocarbons in Italy", Layman's Report, After-Life Plan and "Environmental Guideline for contaminated soil bioremediation." (English, French, Spanish versions)*

Policy impact

Soil Thematic Strategy (COM(2006)231)

Project contribution: supporting the second objective which is focused on restoring degraded soils to a level of functionality consistent with the intended use, also considering the cost implications of the restoration of soil. The project develops a sound solution for the restoration in situ of contaminated soils by oil pollutants through bioremediation approach, but it also addresses the socio-economic aspects of the remediation actions by proving their cost-effectiveness and scalability.

EU Biodiversity strategy for 2020 (COM(2011) 244 final)

Project contribution: supporting the Target 2 related to the restoration of the degraded ecosystems, by providing effective solutions for the in situ bioremediation of contaminated soils by oil pollutants, hence providing for the necessary conditions for enhancing biodiversity and restoring ecosystem functions.

Seventh Environment Action Programme (7th EA")

Project contribution: support the priority objectives of the 7th EAP, related:

- safeguard the Union's citizens from environment-related pressure and risks to health and well-being, by providing a feasible solution for the issue of soils heavily contaminated by toxic compounds)
- turn the Union into a resource-efficient, green and competitive economy, by proposing a cost-effective working protocol to bioremediate soils that valorize waste biomass

The first richness coming from LIFE BIOREST project is the microbial biodiversity isolated from this extreme environment. As globally recognized, it is more and more important to protect plants, animals and microorganisms, and the terrestrial, marine and freshwater ecosystems of which they are a part. Biodiversity is essential for our existence and intrinsically valuable in its own right (Environment Protection and Biodiversity Conservation Act 1999, EPBC Act, the Australian Government). This is

because biodiversity provides the fundamental building blocks for the many goods and services a healthy environment provides. The EU is also committed to the protection of biodiversity by launching the EU Strategy to limit the loss of biodiversity and the degradation of ecosystem services in the EU by 2020. It stands to reason that we cannot waste all the efforts taken during LIFE BIOREST. UNITO wants indeed to respect it and currently is storing all the fungi of Fidenza site in its Fungal Collection (Mycotheca Universitatis Taurinensis, www.mut.unito.it). Since 2008, the MUT has been affiliated to the World Federation Culture Collections (WFCC), which represents a global network aimed to the ex-situ conservation of microbiological biodiversity.
Moreover, since 2008, MUT has become a member of the European Culture Collections' Organisation (ECCO). Fungi are stored in a different condition, targeted on the peculiar development behaviour of each fungus. For instance, long time conservation is made by means of lyophilisation or cryopreservation. After the procedure, each strain is re-activated to assess the stability of the preserved fungus. This is repeated during the time to be sure always to possess the original strain.
Moreover, all the strains are listed in a public database with a unique recognizing code. All the information about the growing capability and medium preferences, the biotechnological application etc. are included in the fungus datasheet.
This is free for consultancy by any academic and institutional entities as well as industries potentially interested in using fungi in different applications. In the case of actual industrial exploitation, some issues may have arisen regarding the intellectual and economic properties of these strains. This is why MUT is a profound supporter of the Nagoya Protocol. The Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization (ABS) to the Convention on Biological Diversity is a supplementary agreement to the Convention on Biological Diversity. It is aimed to establish the fair and equitable sharing of benefits arising out of the utilization of genetic resources. Its objective is the fair and equitable sharing of benefits arising from the utilization of genetic resources, thereby contributing to the conservation and sustainable use of biodiversity. Legal certainty and transparency for both providers and users of genetic resources are the main goals. MUT applies these principles in running the Fungal Collection, in all the collaborations with industries and academic partners, etc. and it is going to do as well with the fungi isolated from the Fidenza site.
UCSC is also storing all bacterial isolates from the project activities in its collection, and its planning to screen at least part of them for the degradation of other pollutants and for other properties of interest such as plant growth-promoting activities.

6.3 Analysis of benefits

Environment benefits

Soil chemical contamination is still a largely unsolved problem at global, European and regional levels. European data show that there are about 5.7 contaminated sites for every 10,000 inhabitants; number rising to 42 if potentially contaminated sites are also considered.
The statistics updated to 2014 estimate the number of sites to be reclaimed in Europe at 340,000 of this only 15 % has been reclaimed or is undergoing reclamation (Van Liedekerke et al., 2014). Heavy metals represent the main form of contamination, with 35% of the sites involved; followed by linear hydrocarbons (24%), polycyclic aromatic hydrocarbons (PAH: 11%), BTEX (benzene-toluene-ethylbenzene-xylene: 10%) and chlorohydrocarbons (8%). These last four categories together form the class of hydrocarbons, and their summation leads to 53% of the total contaminated sites in Europe.
PAHs are a group of persistent, semi-volatile organic pollutants. They accumulate in soils because of their persistence and hydrophobicity and tend to be retained in the soil for long periods of time. The

most frequent ones are anthracene, fluoranthene, naphthalene, pyrene, phenanthrene and benzopyrene. The very low water solubility of PAHs and the slow mass-transfer rates from solid phase may limit their availability to microorganisms, thus hindering natural attenuation by autochthonous microflora. The situation in Italy and Emilia Romagna reflects European data, with hydrocarbons representing the main type of soil contaminants.

Hydrocarbons can be degraded to structurally simpler molecules: at best, the complete mineralization of pollutants can be achieved; otherwise, it is important to verify that the disappearance of pollutants is not associated with the formation of metabolic intermediates with even greater toxicity. The term bioremediation indicates the use of microorganisms or plants to remove or immobilize the contaminants by lowering the toxicity of the system. Specifically, it distinguishes between biostimulation, when nutrients (mainly N and P) are added to the soil to restore optimal ratios for the growth and activity of microorganisms, and bio-augmentation when selected strains with marked degradation capacity are added to the soil to be reclaimed.
In recent decades, the effectiveness and economic convenience of bioremediation have been widely demonstrated (Azubuile et al., 2016). Nevertheless, the diffusion of this technique at European and national level is still limited: in situ biological treatment is applied in less than 20% of cases and with different percentages in European countries (Van Liedekerke et al., 2014).
There is, therefore, an important gap between the scientific progress achieved in the last decades in terms of bioremediation and the application of this technique. For this reason, the European community financed the LIFE BIOREST project, with the aim of demonstrating the advantages of an application-based remediation protocol of appropriately selected bacteria, fungi and plants.

In the LIFE BIOREST project, through the development of an innovative miniaturized screening system, the best performing strains were selected in terms of biodegradation of pollutants, biosurfactant production and redox enzymes. Thirty fungal and bacterial strains were selected for which, thanks to the participation of an industrial partner, the industrial inoculum production and the introduction into contaminated soil were optimized. The strains of fungi and bacteria were analysed individually and in different combinations (consortia) to verify the ability to reduce the concentration of different pollutants over a two-month period through the preparation of microcosms containing several hundred grams of contaminated soil. The 6 most promising fungal and bacterial consortia were used to set up a series of mesocosms of 15 kg of soil: during the current test, the ability to remove pollutants and reduce soil toxicity is monitored. The best microbial consortium was applied in a biopile at the Fidenza site to treat about 350 m^3 of contaminated soil. The soil of the mesocosms was also used to select among the genotypes of 20 species of plants the most suitable for the revegetation of the ground reclaimed in the biopile, creating a demonstration area accessible to the public.

The chart here shows the removed hydrocarbons (TPH, mg/kg of soil) by the bioaugmented biopile (LIFE BIOREST) and the not-bioaugmented one (control) during time. The bioaugmentation led to faster pollutant degradation. LIFE BIOREST biopile removed up to 300 mg/kg of hydrocarbons already after 60 days. At the end of the treatment, it was more efficient: TPH removal was 38% higher than the control. The toxicity was even halved in comparison with the control.

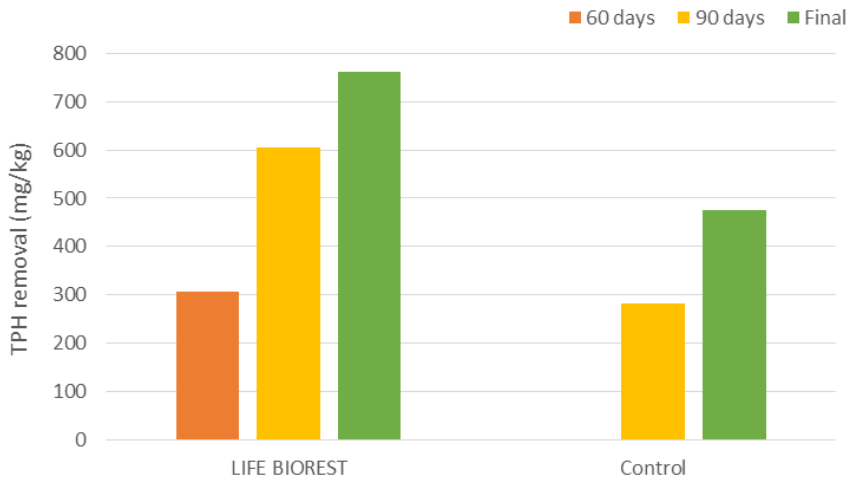

Fig. 19 *LIFE BIOREST bioremediation results compared to the traditional one.*

Economic benefits

The Global Soil Treatment Market was accounted for $26.52 billion in 2016 and expected to grow at a CAGR of 9.2% to reach $49.25 billion by 2023 (Source: strategymrc.com). Therefore, the soil protection segment is anticipated to be one of the most significant market segments, in terms of value, in the upcoming years. North America accounts for the largest market share, followed by Europe. The Asia Pacific is the fastest-growing market owing to rapid growth in the agricultural sector. Specifically, contaminated soils represent a growing challenge. Greener and sustainable solutions able to treat polluted soils and at the same time, reestablish its ecological functions represent the prerogatives of the growing market demand.

Globally, hundreds of thousands of contaminated sites have been identified and require clean-up. Therefore, soil remediation is becoming a significant driver of technology, products and services.
According to the state of EU soil pollution, in 2014 less than 15% of the contaminated soils have been remediated, and only 5% of the remediated soils have been treated with biological methods (Van Liedekerke et al., 2014). In last five years, the number of sites that require remediation is further growing and 2019, according to the last report of the Joint Research Centre of the European Commission, 650,000 contaminated areas have been registered in the inventories of the 28 Member States (Status of local soil contamination in Europe: Revision of the indicator "Progress in the management contaminated sites in Europe, JRC 2019). Primary contaminants present in soil are heavy metals, mineral oils, volatile organic compounds and polycyclic aromatic hydrocarbons that require site-specific protocols and approaches.
Technologies to remediate contaminated soil fall into two principal clean-up approaches: *in*-situ (on-site), or *ex*-situ (on- or off-site). *In*-situ treatment deals with contamination without removing material from the ground. *Ex*-situ treatment requires the removal of contaminated soil for treatment or landfill. This being said, there is a growing preference for *in*-situ approaches, and for niche technologies for specialized chemicals, which cannot be treated by other remediation technologies. Biopiles are usually, 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.
Several conditions have to be considered to make the process efficient and cost-effective parameters, such as temperature, oxygen, pH, aeration and nutrients. The biopile is one of the most used among bioremediation techniques mainly for cost-effectiveness, even though many parameters have to be controlled to establish effective biodegradation (Whelan et al., 2015).

By adopting an **integrated biological approach** (microorganisms and plants) with the combination of bioaugmentation and biostimulation approaches, the LIFE BIOREST project contributes to place the partners in a prominent position for the potential commercial exploitation of the results.
The **cost-efficiency** of the LIFE BIOREST approach was guarantee thanks to the design of a protocol for the production of cheap microorganisms and suitable for bioremediation treatment of 530 tons of soil with more efficiency in the degradation of contaminants. This approach ended up in shorter biopile running times, with consistent energy and management costs saving. The economic impact has been proved according to the average biopile cost per ton (150 €/ton of which 110 €/m3 are for running operations), costs of the bioaugmented bacteria production (2-20 €/ton) and the time of treatment.
As regards the treatment of PHAs, the LIFE BIOREST methods demonstrated a reduction of 50% of the time of treatment (3 months vs 6 months) which translated in up to 30% reduction of the overall costs of soil treatment.

The bioaugmentation led indeed to faster pollutant degradation compared to the traditional approach (the portion of biopile without selective bioaugmentation). The LIFE BIOREST biopile removed up to 300 mg/kg of hydrocarbons already after 60 days. At the end of the treatment, it was more efficient: TPH removal was 38% higher than the control. The toxicity was even halved in comparison with the control (a diagram of removal time-efficacy is included in the Layman's and the Final Report).

As a final consideration, remediation has to be tailored on each single intervention situation. Therefore, an endless number of case studies apply rather than “traditional remediation techniques”. In LIFE BIOREST project we have validated a protocol which is:

- Potentially applicable worldwide to improve existing remediation approaches able to treat PHAs and BTEX polluted soils
- Economically sustainable and integrated into remediation activities with a less impact
- Bringing a reduction of the time of recovery of the contaminated land
- Eco and socially-friendly

Social benefits

Science, technology and innovation in the environmental field play an increasingly crucial role in the future of our society. In order to effectively carry out initiatives to protect and enhance the environmental heritage, promote knowledge and implement sustainable development projects, citizens must play a strategic role in the development of suitable social and economic conditions, science and environmental technologies and expertise. These, on the other hand, look like still today very often dispersed in the environment is often seen *rather* like a *complex* system of problems and limitations *than* as an opportunity for growth and development. Important surveys (European Commission (2014) – Special EUROBAROMETER 416 “Attitudes of European citizens towards the environment”) in the field show a strong gap between scientific knowledge, available methods and tools and collective awareness of their importance and their implications for the development of sustainable growth.

The socio-economic evaluation performed by LIFE BIOREST was included in the Action C1, devoted to the monitoring of impacts generated by the project during its lifecycle.
The first part of the socio-economic study has allowed understanding elementary environmental awareness, consistency between such awareness and sustainable behaviours and perceived environmental concerns by citizens. The socio-economic impact evaluation was carried out by **surveys** handed out to the target audience identified during public events and communication actions, which were also aimed at promoting project objectives and showing on-going activities and results.
Interviewees were divided into three primary target audiences: (1) general public, with particular attention to secondary and higher education students, (2) university researchers and entrepreneurs operating in the biotech, green chemistry, and remediation sectors and (3) policymakers and project stakeholders. Data collection mainly concerns the Emilia-Romagna Region (Italy), in particular the city of Fidenza, where the proposed bioremediation treatments took place, and all areas where research activities were carried out by LIFE BIOREST partners (Italy, France, Spain).
The survey was distributed in the period March-October 2017, during 14 events organized and attended by LIFE BIOREST partners. In this experimental period, **750 surveys and 5.548 responses** were collected. Overall, **air pollution** is considered the most serious of environmental emergencies by 38% of total interviewees, followed by **water pollution** (23%), **waste generation** (20%) and **soil pollution** (19%). The general public has no doubt about the greatest impact of air pollution (40%) and consider soil pollution last in order of harmfulness (16%). Instead, researchers, entrepreneurs and policymakers consider waste the most severe environment threat (35% and 34%) (***Fig. 20***).

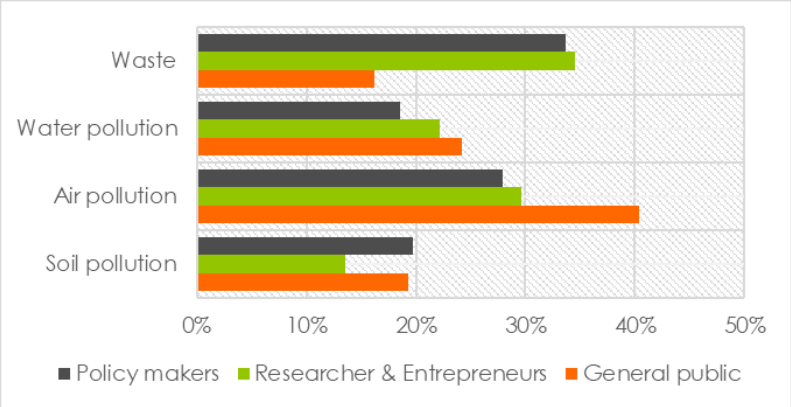

Fig. 20 *Environmental emergency perception for each of the three survey target audiences*

Overall, more than a half of interviewees attribute the greatest responsibility for the poor spreading of biological remediation methods to the need for high investment (35%) and the complexity of administrative and regulatory procedures (23%).

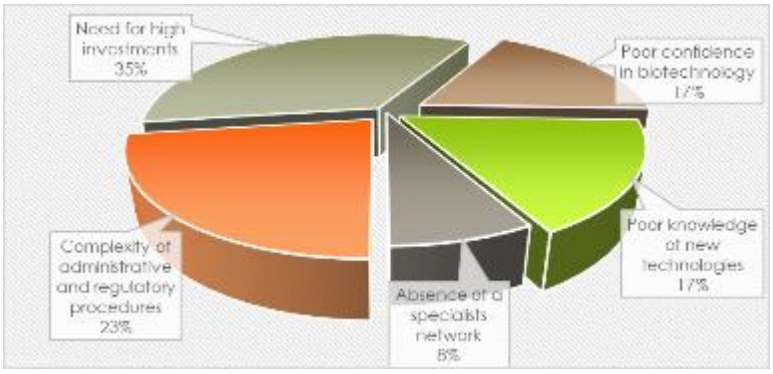

Fig. 21 *Bioremediation: barriers to widespread use.*

Looking at the three samples of the interviewed audience separately, uniformity of opinion between the general public and researchers and entrepreneurs can be observed.
For these targets, demand for high investment represents the main obstacle to overcome (36% and 32% respectively) to ensure the wider diffusion of biotech methods in reclamation, followed by ineffective legislation (22% and 28%), while third is the poor confidence in biotechnology (17% and 26%).

Instead, policymakers wish to be more informed on technologies and innovations (31%) to support policy-making actions towards more sustainable solutions in the reclamation sector (33%).

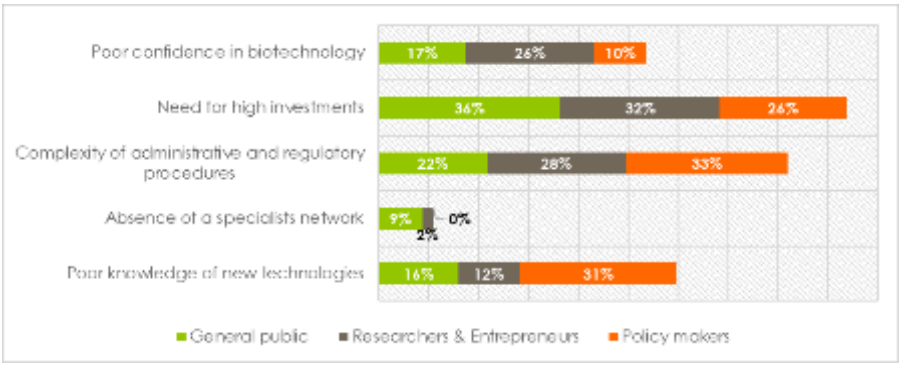

Fig. 22 *Main barriers to overcome for a wide use of biotech solution in soil remediation, according to each target audience*

Biotechnology and phytoremediation are considered the most promising remediation techniques for about 80% of the interviewed samples, even though they constitute a minimum percentage among solutions used for soil decontamination.
The results obtained through this survey suggested the need to strengthen public awareness on soil pollution.

Results obtained by the LIFE BIOREST socio-economic study contributed to define a better communication strategy able to foster knowledge about the soil pollution and benefits of the bioremediation approach among the general public, policymaker, academia and industrial sectors. LIFE BIOREST information and education campaigns were developed by involving high school students as "BIOREST ambassadors", to enhancing the "LIFE BIOREST Bioremediation method" and promote sustainable use of resources.

Local communities were the primary target audiences of these communication campaigns, who are often worried but also not correctly informed about the real risks related to soil pollution and the possibilities offered by modern technologies to address and solve them.
Thanks to the "LIFE BIOREST ambassadors" initiative, more than 55 videos were produced and disseminated via social networks, reaching 191.619 people.

Replicability, transferability, cooperation

Besides ongoing and upcoming soil and water remediation requirements, the soil treatment market is driven by different factors such as growing organic food market, demand for high-quality food, and shrinking arable land. The approach developed in the LIFE BIOREST project is somehow going beyond the bioremediation of organic pollutants. Indeed, considering the potential of microorganisms, the same strategy could in the example be adopted in improving soil fertility, in speeding up the shifting of soils from traditional to organic agriculture, in preventing (by ready to use protocols) pollution by "at risk" industrial activities. Therefore, LIFE BIOREST has the potential to integrate into several of the different policies of the EU. In conclusion, from the social point of view, contaminated soil represents a lost economic opportunity and a threat to the health and wellbeing of human beings and the environment. This contamination is the legacy of industrialization and insufficient environmental legislation and enforcement. LIFE BIOREST is the legacy of an approach, which we ideally want to export worldwide. For this reason, dissemination of the results is of fundamental importance in the LIFE BIOREST project.
The project has demonstrated to date that the bioremediation with bioaugmented and biostimulated microorganisms is cheap and environmentally sound. With the technologies available to date, we foresee an application of the developed method also to the deep soil which could eventually avoid removal and mechanical mixing.

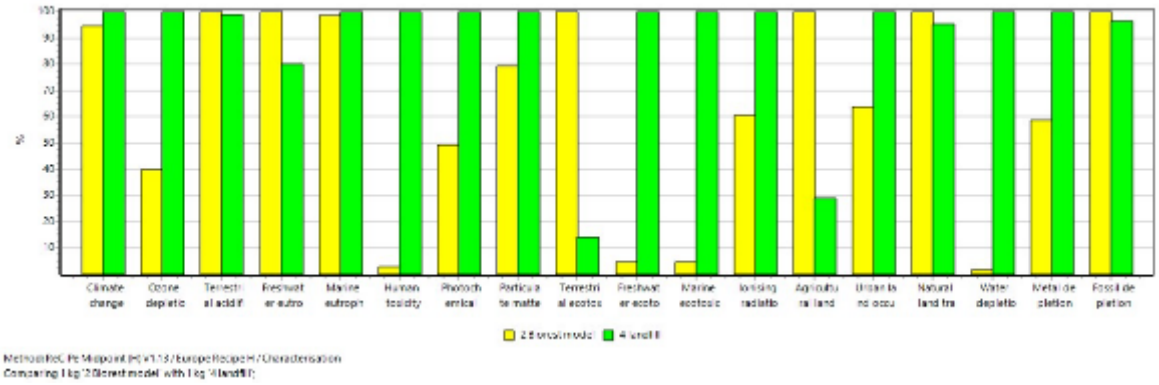

Fig. 23 *Impacts assessment for LIFE BIOREST model (yellow indicators) and landfilling (green indicators) calculated according to ReCiPe midpoint (H) V 1.12 method*

More precise and quantitative data was produced at the end of the project. As an example of the efficacy of LIFE BIOREST approach in figure ***Fig. 23*** are reported the estimated environmental impacts due to the treatment of contaminated soil by biotreatment and bioaugmentation approach compared to the option of disposing of soil in a landfill. The calculation is conservative, considering a mild remediation efficiency in LIFE BIOREST and a total efficacy of sanitary landfill to prevent emission to soil and water bodies. Mid and endpoint analysis are reported (***Fig. 24***).

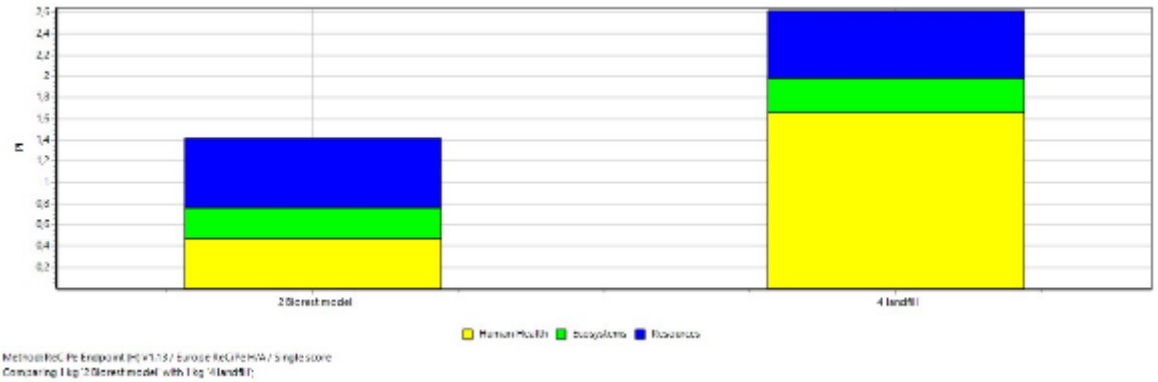

Fig. 24 - *Impacts assessment for the LIFE BIOREST model (left) and landfilling (right) calculated according to the ReCiPe endpoint (H) V 1.12 method*

Finally, considering the important environmental indicators listed in **Table 4**, the restoration of soil with a LIFE BIOREST like-model is highly recommended because it decreases the impact on population health and the environment when compared with no action scenarios and soil landfilling.

Table *Environmental indicators*

IMPACT CATEGORY	UNIT	LIFE BIOREST MODEL	LANDFILL	NON ACTION
Human toxicity, cancer	cases	3.21E-09	1.04E-09	1.74E-06
Human toxicity, non-cancer	cases	4.35E-10	8.03E-11	2.43E-07
Freshwater ecotoxicity	PAF.m3.day	1.44E+00	2.49E-02	4.46E+02

The **LIFE BIOREST approach was replicated at the mesocosm level on other French, and Spanish hydrocarbon contaminated soils**. Regarding the French soil, the set-up operations took place at the Laboratoire Chrono-environment of the Université de Bourgogne Franche-Comté (Montbeliard, France) with the collaboration of Professor Michel Chalot and Instituto de Recursor Naturales Y Agrobiologia de Sevilla (Sevilla, Spain) with the collaboration of the CSIC partner.

The mesocosm experiments were performed in triplicate and consisted of 3 x 4 kg pots of contaminated soil (12 kg total) supplemented with rice husk (as a carrier) and treated with the selected consortium of fungi and bacteria (consortium A) produced as described in deliverable B2.D3 (Protocol for sustainable large-scale production and functionalization of biocatalysts) and already applied to the biopile of Fidenza SIN. The abiotic control replicates are the soil + rice husk carrier without microbial inoculum. Chemical, bioavailability, ecotoxicological and microbiological analysis were performed on both European soils. The total polycyclic aromatic hydrocarbons (PAHs) content was extracted for both French and Spanish polluted soil, and PAHs concentration was measured in control (pots only with polluted soil) and treated (pots with polluted soil plus bacteria and fungi consortia) at different sampling times (0, 20, 60 and 90 days).
The French replication test indicates that the total PAHs content did not change over time. However, in the case of the Spanish replication test, the total PAHs concentration slightly decreased.

The bioaugmentation with the selected strains was not as performing as in the soil of the Fidenza SIN. This may seem contradictory but was indeed expected. LIFE BIOREST is indeed based on the importance of building up a system designed on a case by case basis. When we stated that strains isolated in the polluted site were possibly adapted to it, the meaning was the adaptation to a plethora of variables they

were used to face, for instance, the pollutants mixture and concentration, but also the pH of the soil, and other physicochemical factors. These parameters are essential and may interfere with the replicability of a specific consortium in another soil. Autochthonous microorganisms are, therefore, the critical factor to have a bioaugmentation system working.
Besides, the lack of biodegradation after bioaugmentation of the French soil, as evidenced by negligible changes in PAH concentrations, could also be predicted well by independent bioavailability measurements. The initially low bioavailability exhibited by PAHs in this soil that did not change after treatment was consistent with the observed recalcitrance, by indicating an extremely low bioavailability of the pollutants, that would eventually resist the potential of the inoculated consortium to enhance bioavailability and reduce the residual levels.
According to the mesocosm trials results, it is possible to assert the **validity of the LIFE BIOREST bioremediation approach** and at the same time the **role and importance of a tailored protocol** based on the selection of autochthonous microorganisms that naturally populate the contaminated site.
This assumption is in agreement with an activity performed by UNITO, using the expertise acquired in the project. During a project funded and run at a regional level, the goal was to treat polluted urban areas that are used as gardens. Much effort was dedicated to the isolation of the autochthonous microorganism of the soil. This approach paid since in situ fungal treatment led to complete removal of pollutants.

LIFE BIOREST participated in the conference "Rehabilitation of lacunal areas within contaminated sites" Mantova – Italy October 18th, 2018, organized by the Municipality to share good practices and sustainable models' decontamination of polluted soil.
In Mantova, there is the Site of National Interest "Lakes of Mantua and Chemical Pole" recognized and included in the National Reclamation Program with Law 179 of 13 July 2002, about the characteristics of the site, the quantity and danger of the pollutants present.
The characterization activities allowed to analyse the state of contamination of the soil and water of the Site of National Interest; except for some areas not yet characterized or in which the characterization must be completed.
The data obtained showed substantial soil contamination due to aromatic organic solvents, organo-halogenated solvents, light and heavy hydrocarbons, MTBE, heavy metals and locally also PCBs and PCDDs / PCDFs. In 2008, the characterization activities of the lake and river areas included in the SIN were completed, as well as the analyses carried out on sediments, waters, benthos, bivalves and fish.

Lesson learned

The lesson learned was described in detail reported in the Layman's report. It was targeted at a non-specialist audience and resulted in an informative tool for decision-makers and non-technical parties of the project objectives and results. It represented, along with the project website, one of the main tools for disseminating information after the project's end.
The report included an overview of the soil pollution problem and its consequences on the ecosystem, human and animal's health, offering the alternative of the bioremediation based on the approach developed by LIFE BIOREST project, to restore polluted soil affected by hydrocarbon's contamination.
Objectives and actions of the project are reported, along with the benefits of the innovative solution proposed by LIFE BIOREST.
The results of the project were described in a non-technical way to reach a wider audience, including non-technical parties.

In general terms, the lesson learned from LIFE BIOREST bioremediation approach has to be tailored on each single intervention situation. Therefore, an endless number of case studies apply rather than "traditional remediation techniques". In this project, the bioremediation protocol was proven:

- Potentially applicable worldwide to improve existing remediation approaches able to treat PHAs and BTEX polluted soils
- Economically sustainable and integrated into remediation activities with a less impact
- Bringing a reduction of the time of recovery of the contaminated land
- Eco and socially-friendly

UNITO activated side research activities aiming at deepening the information on fungi and their metabolites. First, biosurfactants demonstrated to be powerful molecules with several biotechnological application. At the moment, the production cost and their safety are still an issue, which is ultimately limiting their application, for instance, in environmental fields. It is important to define new microbial sources that may produce very active biosurfactants which could be safely disposed of in polluted soil to help remediation techniques as in other industrial activities. During LIFE-BIOREST project, it emerged that most of the molecules have a bacterial origin: despite bacterial fermentation are not so tricky, the upstream of intracellular products is very difficult and costly. The actual possibility to apply bacterial biosurfactants has some drawbacks indeed. However, fungi demonstrated to express extracellular molecules with both emulsification and surfactant activities. The LIFE-BIOREST outline and aims were not focused on these molecules but UNITO decided to spend additional efforts on this. Several fungi were chosen among the here isolated strains, but including also other strains coming from MUT Fungal Collection. The MUT activate a collaboration with the Department of Chemistry to increase the information about the compounds secreted. The production protocol was developed from the protocol of LIFE BIOREST.

As regards the analysis, qualitative information was here implemented with quantitative ones: in collaboration with the chemist's group, surface tension analysis, CMC and the contact angle were measured. The idea is to screen vast fungal biodiversity and to select the most promising one. Surprisingly some fungal metabolites were highly performing, posing for new questions: what are the produced molecules? The actual chemical nature of these molecules is the obvious step forward. In collaboration with ACTY and the University Federico II of Naples (Italy), joint scientific researches have begun, aiming to identify the fungal biosurfactants.

The protocol applied for biosurfactants production foresaw the use of oil as sole carbon source. Some fungi were not only capable of producing biosurfactants but did also show a very strong growth. Indeed, it seemed clear that some fungi were capable of activating a primary metabolism that exploited this peculiar nutrient as more simple one as glucose. The idea that LIFE BIOREST isolated strains had very good skills in term of nutrient consumption and how to define a nutrient for them was already evident during the project (e.g. pyrene used as well as glucose). They are indeed capable of triggering a secondary metabolism including enzymes that guaranteed the exploitation of a source as the olive oil. This information was very useful during the congress held in Bologna (ICEEM07) where there was the possibility to share scientific achievement and prospects with other researchers working in the field, including a group of the University of Modena and Reggio Emilia. They are working on biofuels production, but they were looking for new bio-catalysts. UNITO activated a collaboration with them (prof. Luisa Barbieri and prof. Luca Forti) to study more in detail the enzymes involved in the olive oil transformation because potentially including lipases and esterases. Lipases (EC 3.1.1.3) are gaining growing importance as demonstrated from a global market prevision of $590.5 million by 2020. Following proteases and amylases, lipases are considered as the third group in the volume of sales. The rising demand for these enzymes is amenable to their great potential for present and future industrial

applications in many fields (textile, leather and paper industries, biofuel, detergents and cosmetics). These enzymes are indeed very important in the transesterification of oil and lipidic substrates to ultimately produce biofuels.

The collaboration merged the scientific skills of MUT in the fermentation protocols and biodiversity studies with Barbieri's group on transesterification reaction and products upstream. During the project, almost 200 strains were studied, including also many LIFE BIOREST isolated strains. First qualitative screening was run with olive oil and trybutyrin as sole carbon source. The screening allowed the selection of almost 50 strains that were then tested in liquid fermentation thanks also to the standardized lipase kinetics protocol that was developed by UNITO in the meantime. Submerged fermentation highlighted considerable lipases production in few strains which were capable of obtaining good enzymatic yields in a medium supplemented with a complex matrix like olive oil as sole carbon source. Scale-up experiments highlighted the good performances of *F. solani* which was capable of producing lipolytic enzymes comparably to results achieved in small flasks. *F. solani* lipases (FsLIP) showed broad pH stability, while activity was stable at 40°C. FsLIP showed transesterification reaction, observable on silica layer by triglycerides spot reduction. A reduction of about 30--35% of triglyceride spot area occurred after 72 hours of enzymatic activity. Implementation of biochemical characterization could provide more info regarding potential application in various biotechnological fields.

Innovation and demonstration value

Soil pollution is a huge issue worldwide, including Europe. Several different, often intriguing, technologies have been studied, but very few of them have been applied at large scale.
The main concerns are often related to the economic and environmental sustainability of these processes. Many researches did not consider the feasibility of a process from the very beginning: unfortunately, many technologies that provide good results at lab scale do not confirm themselves at a larger scale or are abandoned because of not-applicable in real environments.

LIFE BIOREST showed an innovative approach from the very beginning, which may have helped to reach the proposed objectives. First of all, despite at the beginning, some part of the research was done in laboratories, it was always driven by the final actual application that the project was aiming for. In any step, the best performers were selected, and they indeed were able to work in the *in situ* treatment, in axenic and poorly controlled conditions. Moreover, thanks also to the precious collaboration between Academia and Industries, the technical and economic feasibility of the method was discussed a challenged in any step of the project. This clearly allowed to develop a sustainable method, that can compete with the traditional one also from an economic point of view.
At a glance, the use of microorganisms to degrade pollutants cannot be considered a new approach, but we need to balance the word 'microorganisms' carefully. Indeed, most of the researches are dealing with bacteria. Most of the microbiologists in the world are bacterial specialists. Fungi is a less known world. From one side, most of this Kingdom has been barely studied.
Up to now, it contains around 90,000 species, and thousands are described every year, is estimated that there might be more than one million fungal species in the world (Zaragoza 2017). Moreover, there are very few institutions that work with them and are specialized in their conservation and characterization. Thanks to the presence in the consortium of UNITO, LIFE BIOREST proposed then an innovative approach based on the synergic activity of both bacteria and fungi, whose activities can complete each other. The other limitation of many studies is that they are focused on a single compartment of the biological sciences. Bio-based treatment should not be in antithesis. Mycoremediation should not be an absolute alternative to phytoremediation. LIFE BIOREST looked at nature in its complexity and merged

different expertise to integrate microorganisms and plants together. The successful demonstration of this eco-friendly bioremediation technique was first tested in microcosms and mesocosms, where all parameters for field implementation were optimized. This bio-based multifaceted approach was then validated at *in situ* levels where it proved to be a highly performing bioremediation technique Zaragoza, O. (2017). Mycology.

Policy implication

In Italy, the absence of a national register of the state of contamination and reclamation of polluted soils by hydrocarbons is the reason for a study carried out by the ITB, consisting of an extensive mapping of contaminated sites classified by each Italian region.
The study examined **20 Regional registries**, to highlight the impact of oil pollution on the national basis and the state of restoration of the contaminated sites.
More than **20 thousand sites** of **regional interest have been surveyed**, of which **9,487 still require remediation or further investigations**, within this category, there are **2,119 sites contaminated by hydrocarbons (oil and derivatives)**, equal to 22% of the total contaminated sites in Italy. The 10,560 remaining areas included in the registry are generally classified as not polluted after assessment and potentially polluted, which therefore require analysis and further verification.
The objectives of a common database are aimed at sharing a model to construct a complete picture of the state of soil pollution in Italy, able to be updated according to a unified data exchange standard. The proposed database should contain information on the progress of the management of the sites, potential sites, primary contaminants present, type of activities of the contaminated (according to the EIOnet scheme), used cleaning technologies, costs (public and private).
The study is focused on creating a geo-referenced picture of the sites contaminated by hydrocarbons with the aim of evaluating their application potential with respect to the bioremediation model proposed by LIFE BIOREST. This study has led to the publication of the report "**Soil contamination from hydrocarbons in Italy – A mapping of progress in the remediation of Sites of National and Regional Interest**", distributed for the first time during the Workshop "Strategie Green per le città del Futuro" at Opificio Golinelli (Bologna) on 10 May 2019. Thanks to actions to promote and disseminate the results obtained from the project, this study was aimed at informing the large and restricted public about the benefits of biological bioremediation technologies as an alternative to traditional landfill disposal practices for hazardous waste. It aims to sensitize the Municipalities, Regions and Provinces that have contaminated sites on their territory (both 42 SINs and SIR) to the objective evaluation of bioremediation techniques.

The second part of the LIFE BIOREST socio-economic study was focused on mapping the state of pollution and the main obstacles to the full application of the bioremediation method. A database of all the sites of regional and national interest has been developed starting from 20 registries produced by the Italian regions. The database represents the **first national instrument for the census of all polluted sites in Italy**, where they were classified by region, source and type of contaminants with a special focus on hydrocarbons pollutants and state of implementation of reclamation. **Over 20.000 Italian sites were mapped, of which 9,487 require remediation interventions**. This category includes 2,119 sites contaminated by hydrocarbons (oil and derivatives), equal to 22% of the total number of contaminated sites in Italy. The resulting report was distributed for the first time during the Workshop "Strategie Green per le città del Futuro" Bologna, Opificio Golinelli 10 May 2019. The study was based on the data made available by the Regions, regional agencies (ARPA's and APPA's) and by the Ministry for the Environment. Given that such data are sometimes outdated or lacking information on the type of

pollution, the study must be held as preliminary. Indeed, a number of potentially contaminated sites still need characterization. The information on the Sites of Regional Interests has been extracted from the 20 regional registers provided for in the environmental regulations. The regions with a greater concentration of hydrocarbon-contaminated sites are Lazio (510), Tuscany (338), Sardinia (219) and Lombardy (177), while only a few of them are found in Valle D'Aosta (3), Abruzzo (44) Umbria (50), Campania (60) and Marche (69). The characterization tests performed at the Sites of National Interest highlighted a predominance of pollution by a combination of heavy metals, chlorine compounds, hydrocarbons, pesticides and herbicides, which on the whole account for 61% of the various contaminants.

As the final part of the study, the Environmental Guideline for contaminated soil bioremediation was elaborated. It represented the final publication of LIFE BIOREST project produced in three languages – English, Spanish and French, considering a useful tool for the public authorities and decision-makers who are facing the problem of managing a polluted hydrocarbon site all over Europe. This publication has the objective of presenting to the local authorities the collected and elaborated recommendations and results obtained through the LIFE BIOREST project.

Fig 25 *– LIFE BIOREST publications*

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

The Bioremediation Guidelines could be then become a useful tool for the policymakers by also enlarging their knowledge about all the possible solutions for reducing pollution of soil and water. They cannot be considered a procedural protocol that can be applied anywhere, because unfortunately, any site has its features and characteristic. As stated above, the method can, however, be implemented and adapted to different problems and considered at the beginning of a site management plan.

The bioremediation model that has been developed during the project offers several advantages also in terms of replicability in other polluted sites. We know that the solution to pollution cannot be unique, but it's site-specific. LIFE BIOREST major achievements are surely the development of a bio-based treatment and its validation in a site at large scale, but mostly to set up of a methodological approach that can be replicated in many different sites. It is indeed a flexible approach that can be modelled

accordingly to the environment conditions, the pollution level, the contaminants, the matrices, etc. For instance, a similar approach was used to treat the soil of an urban garden that was polluted by hydrocarbons. Similarly, the mycoflora of the site was isolated and selected for its degradation skills. The method was implemented with other pollutants of interest (i.e. perylene) and with other achievements to be reached (soil ready to be used for agriculture purposes). But it did work. The fungal bio-augmentation led to the removal of much higher pollutants than the control. Moreover, the project foresaw also the study of a bioactivation approach, based on the addition of an organic soil conditioner to provide nutrients and microelements to the soil in order to activate the microbial community of the soil. This treatment helped the soils gaining a small reactivation of its ecological services, but the degradation rate was significantly lower than the inoculation of selected strains which are the actual degraders.

Since the LIFE BIOREST approach gave such promising results, it has been proposed in other projects that have been submitted. For instance, ATLANTIS project (CE-BIOTEC-08-2020) has the goal to treat polluted wastewaters and sludge. The first Action of the project would study the microbial community of these polluted environments. It surely would have some peculiar adaptation skills that may help in the bioremediation.

Regarding the cooperation initiatives promoted by the LIFE BIOREST project, the Summer School held in Torino was a fruitful networking moment where it was possible to discuss about the results and possible afterlife projects with speakers and participants. More in detail, UNITO and prof. Simona di Gregorio (University of Pisa) are now working on projects that have some overlapping activities. We decided to merge our experience in microbial monitoring of polluted soil, evaluating different technological approaches. UNITO has mostly worked with fungi and Next Generation Sequencing. It was decided to spend additional efforts to compare these kinds of results with those coming from a novel technology, recently purchased by the prof. di Gregorio's group. The approach utilizing the long-read capability of the Oxford Nanopore MinION was mainly developed and applied for rapidly sequence bacterial ribosomal operons of complex natural communities. Very few information are available on fungi. The two techniques have both pro and cons, in term of scientific reliability and economic sustainability. The idea of this collaboration is to compare these two systems to provide information for further project activities and proposals.

Our studies were of particular interest during a collaboration with Domenico Davolos of the "Istituto Nazionale per l'Assicurazione contro gli Infortuni sul Lavoro e le malattie professionali". He was also interested in performing a whole-genome sequencing of fungi with potential issues on humankind and human-related activities. It was decided to start working on two fungi isolated from Fidenza's site: *Scedosporium minutisporum* MUT 6113, *Trichoderma lixii* MUT 3171. Thanks to the promising preliminary results, INAIL and UNITO are going to submit a project to continue the research.

The scientific skills developed and acquired by UNITO during LIFE BIOREST help them also in other projects dealing with polluted soil. MUT won a project (Re-Horti), funded and run at a Regional level that was focused on the remediation on urban gardens in the urban area of Torino. The project foresee the selection of a case study in the metropolitan area where to perform biological approaches to soil remediation. The concept was to apply microbes or plants. MUT was involved for its expertise in fungal management in soil remediation as well as microbial and ecotoxicological monitoring. The system was scaled up to in situ trials of a green area located in the Municipality which was previously used as a urban garden. Due to the presence of pollutants, the project highlighted that it was not a safe practice.

The contamination was less deep and high than Fidenza's site, but we were able to isolate some adapted fungi with a unique metabolism. In situ fungal treatment led to complete removal of pollutants.

UCSC group coordinated by Prof. Edoardo Puglisi is now officially involved in a project commissioned by ERSAF (Lombardia Region Entity for Agriculture and Forestry) on the bioremediation of an area close to the Masetti Dam in Mantova, Italy. In the project, UCSC together with ACTY from the LIFEBIOREST consortium, an approach similar to the one of LIFE BIOREST is being replicated: degrading microbial strains have been selected from the polluted site, replicated by ACTY and then re-inoculated in the soil, both in the open field and in pot greenhouse trials. Revegetation is also being carried out by the Agronomy group of UCSC of Prof. Stefano Amaducci.

https://video.gelocal.it/gazzettadimantova/locale/a-mantova-batteri-funghi-e-piante-per-bonificare-le-sponde-dei-laghi/98397/98821

http://www.comune.mantova.gov.it/attachments/article/1702/Pieghevole_10x21definitivo.pdf