

# **Education Pack**

# In situ bioremediation





# **Introduction and contents**



With industrial activity and pollution of the environment being widespread, EiCLaR is developing four bioremediation technologies which use the physical, chemical and electrical processes of biological systems (such as plants, fungi and microbes) to break down contaminants and remove pollution from the environment.

Learn more about these novel technologies that aim to make the clean up of contaminated environments more cost effective and efficient. Click links below to go to different pages:

> Types of bioremediation



# Where do the pollutants in soil and groundwater come from?

Pollutants can be natural or man-made and can affect air, water, and soil. They may be from historical pollution incidents such as accidents and spills, contamination from historical industrial land use, contaminant migration overland or by infiltration into the ground, high levels of naturally occurring substances and historical waste deposits such as former landfills. Some common types of chemical pollutants includes substances like industrial chemicals, hydrocarbons, pesticides and metals. Other pollutants include biological, physical and radiological substances.

## What is remediation?



Remediation is the process of cleaning up and restoring sites that have been contaminated by pollutants. These pollutants can contaminate the soil and water on and beneath the site and cause harm to people, the environment or both. Remediation typically uses one or more of the following processes to treat the pollutants: biological, physical, chemical or thermal.





# What is bioremediation?

Bioremediation uses biological systems including plants, fungi, and microbes to break down contaminants and remove pollution from the environment. Microbes are very small organisms, such as bacteria, that live naturally in the environment. Bioremediation stimulates the growth of certain microbes that use contaminants as a source of food and energy.

#### Find out more about bioremediation >

## Bioremediation

A Citizen's Guide to

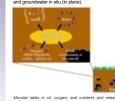
#### What Is Bioremediation?

Bioremediation is the use of microbes to clean up contaminated soil and groundwater. Microbes are verisms, such as bacteria, that live naturally ronment. Bioremediation stimulates the microbes that use contaminants as and energy. Contaminants treated include oil and other petroleun lucts, solvents, and pesticides

#### Jow Does It Work?

Some types of microbes eat and digest contaminant em into small amounts of water and carbon dioxide and ethene. If soil and t have enough of the right microbes, ev can be added in a process called "bioaugmentation

diation to be effective, the right tempera-For bioremediation to be effective, the right itempera-ture, nutrients, and food also must be present. Proper conditions allow the right microbes to grow and multiply—and eat more contaminants. If conditions are not right, microbes grow too slowly or die, and contaminants are not cleaned up. Conditions may be improved by adding "amendments." Amendments range from usehold items like molasses and venetable oil to air micals that produce oxygen. Amendme mped underground through wells to the



The conditions necessary for biore cannot always be achieved in situ, however. At some sites, the climate may be too cold for microbes to be sites, we climate may be too close to the second of microbuse to be active, or the soil might be too dense to allow amend-ments to spread evenly underground. At such sites, EPA might dig up the soil to clean it "ex situ" (above ground) on a pad or in tanks. The soil may then be neated, stirred, or mixed with amendr conditions



To clean up contaminated are drilled to pump some of the groundwater into above ground tanks. Here, the water is mixed with amendments before it is pumped back into the ground The groundwater enriched with amendments allows nicrobes to bioremediate the rest of the cont proundwater underground. Groundwater als pumped into a "bioreactor" for ex situ treatment. Biore-actors are tanks in which groundwater is mixed with microbes and amendments for treatment. Depending on the site, the treated water may be pumped back to the ground or discharged to surface water or to a

How Long Will It Take?

It may take a few months or even several years for microbes to clean up a site, depending on several factors For example, bioremediation will take longer where: Contaminant concentrations are high, or contami-nants are trapped in hard-to-reach areas, like rock fractures and dense soil.

The contaminated area is large or deep.



€°EPA

# What does in situ mean?



When clean up processes occur at the site of the pollution, in the soil or groundwater, this is known as in situ treatment. If these processes are biological, then it is known as *in situ* bioremediation. Conversely, when soil is dug up to clean it above the ground or when groundwater is pumped for treatment above ground it is referred to as ex situ treatment.



# What is the EiCLaR project?



Enhanced and Innovative In Situ Biotechnologies for Contaminated Land Remediation (EiCLaR) was a project funded by the EU and China. It ran from January 2021 until the end of 2024 and was composed of 13 EU and 5 Chinese partners.

The project was coordinated by Professor Timothy M. Vogel at the French National Centre for Scientific Research and Professor Xin Song at the Institute of Soil Science, Chinese Academy of Sciences. EiCLaR has developed scientific and technical innovations for in situ bioremediation technologies that have been developed into industrial processes for the rapid, efficient, cost-effective treatment of a range of environmental pollutants (solvents, metals, hydrocarbons).

These technologies expand the range of applications to industrial sites that contain complex, high concentration pollutant mixtures:

- electro-nanobioremediation
- monitored bioaugmentation
- bioelectrochemical remediation
- enhanced phytoremediation

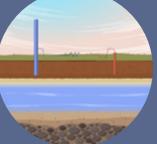


**Electro-nanobioremediation** 

#### Types of bioremediation



**Monitored bioaugmentation** 



**Bioelectrochemical remediation** 



**Enhanced phytoremediation** 



#### **Electro-nanobioremediation**

Electro-nanobioremediation is an innovative and advanced remediation technology that combines three key approaches to efficiently clean up contaminated environments. It integrates electrokinetics, nanotechnology, and bioremediation techniques to tackle complex and persistent pollutants in soil and groundwater.

In this process, electrodes are installed in the contaminated area, and an electric field is applied to promote the movement of charged contaminants towards the electrodes. Nanoparticles, such as zero-valent iron or other metal-based nanoparticles, are introduced to the environment. These nanoparticles help the breakdown of organic contaminants into less harmful compounds.



Electro-nanobioremediation pilot tests



Glycerol-based substrate at the pilot site



Soil extraction from boxes for electro-nanobioremediation column experiments



### **Electro-nanobioremediation (continued)**

Bioremediation comes into play as naturally occurring microorganisms or specifically introduced bacteria take advantage of the enhanced conditions created by the electric field and nanoparticles. These microorganisms can effectively break down a wide range of pollutants, including hydrocarbons, into harmless byproducts, such as water and carbon dioxide.

The combined use of electrokinetics, nanotechnology, and bioremediation in electro-nanobioremediation provides a collaborative effect, leading to faster and more efficient cleanup of contaminated sites. This technology shows great promise in addressing environmental pollution challenges while minimizing the need for disruptive excavation or costly conventional remediation methods.



Substrate dosing unit



Preparation of a new set of power electrodes for regular replacement



Technology demonstration of electronanobioremediation at a site in China



#### **Monitored bioaugmentation**

Monitored bioaugmentation uses microorganisms injected into contaminated soil and wastewater to speed up the breakdown of solvents (e.g. chloroethenes) using the natural biological process of aerobic degradation.

Chloroethenes are used as a cleaning agent in industrial processes and due to improper handling and storage, these chemicals can be released into the environment, where they will sink into the soil and groundwater.

Aerobic metabolic degradation represents a new and promising concept to remove chloroethenes from the subsurface environment. The key benefit of the metabolic aerobic process is there is no risk of formation of hazardous intermediate products and auxiliary substrates are not required and, therefore, with a limited amount of oxygen approximately 100 times more pollutants can be removed as compared to cometabolic degradation.





Isolation of bacteria with target genes

Implementing monitored bioaugmentation technology at a site in Belgium. Injection rig for adding oxygen to ground is shown

If the required microorganisms are not present at a specific site, enriched cultures can be added to the field site (bioaugmentation). Therefore, bioaugmentation in combination with biochar injection and enhanced electrokinetic distribution represents a highly innovative and promising approach developed in EiCLaR. Aerobic chloroethene biodegradation can occur under natural conditions or after addition of oxygen in engineered approaches on-site.



### Monitored bioaugmentation (continued)



Development of lab tests to enhance chloroethene degradation efficiency



Laboratory setup to determine the soil oxygen demand at the test site in Belgium



Cultivation of the aerobic chloroethene-degrading microorganisms



#### **Bioelectrochemical remediation**

In simple terms, bioelectrochemical remediation converts chemical energy into electrical energy by adding an electrode with electroactive bacteria to soil or water to convert pollutants into less harmful substances. Bioelectrochemical remediation technology shares its origins with microbial fuel cells.

The bioelectrochemical remediation system developed within the EiCLaR project leverages bacteria capable of anaerobic hydrocarbon degradation and electron exchange to surfaces or shuttles outside the cell. This well-coordinated biological process converts the chemical energy stored in hydrocarbons directly into electricity. The process involves a series of redox reactions: organic degraders transform complex hydrocarbons into simpler molecules, which are then oxidized by electroactive bacteria in the anaerobic anode. Electrons are transported through conductive materials to a cathode exposed to air, where oxygen is reduced to water.

Bioelectrochemical remediation can simultaneously remove pollutants and recover energy from the substrate. Both electricity generation at the anode and microbial electrolysis, which uses a small amount of energy to drive chosen reactions at the cathode, are utilized.



Large-scale microbial fuel cells experiment. Hydraulic set up (left) and top view of the microbial fuel cell (right)



Microbial fuel cell experiments at the University of Stuttgart



Installing bioelectrochemical remediation technology on a site in the UK



#### **Enhanced phytoremediation**

Phytoremediation is a remediation technique that utilizes plants to immobilize, uptake, stabilize, or degrade contaminants. Enhanced phytoremediation refers to the use of methods and techniques which improve the efficiency of phytoremediation processes (e.g. use of additives, application of electric current).

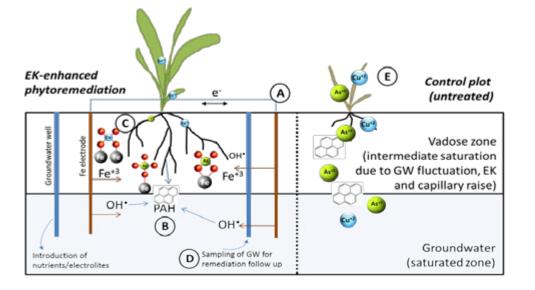
The EiCLaR project went one stage further and developed a novel soil remediation technology to immobilize arsenic and simultaneously degrade hydrocarbons *in situ*. The technology synergistically combines three individual techniques: phytoremediation, chemical immobilization, and electrochemical oxidation. Individually, each method serves a specific purpose, but together they leverage their strengths to effectively address complex soil contamination: phytoremediation utilizes plants to stabilize and degrade contaminants, chemical immobilization binds arsenic to reduce its mobility, and electrochemical oxidation breaks down hydrocarbon. Additionally, the technology enhances soil health and promotes microbial activity, providing an integrated electrochemicalbiological solution for complex soil contamination.

Separate research within EiCLaR has demonstrated that alfalfa and eucalyptus have potential for treating co-contamination by hydrocarbons and chloroethenes.

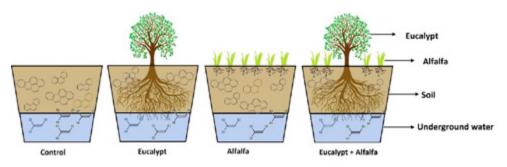


Assessing the potential of alfalfa and eucalyptus for phytoremediation





### **Enhanced phytoremediation (continued)**



Schematic of alfalfa and eucalyptus phytoremediation experiments

Principles of the enhanced phytoremediation approach applied in the EiCLaR project (A) Electrodes are inserted through the vadose zone into the groundwater layer. (B) Organic contaminants will get degraded due to the reactive oxygen species formed by electricity and through excretion of fatty acids (e.g. linoleic acid) by plant roots. Reduced soil toxicity would stimulate indigenous microbial population, which will further contribute to contaminant degradation and promotion of plant colonization. (C) Mobile and bioavailable fractions of metal(loid)s will come in contact with iron oxides originating from corroding electrodes leading to reduced soil toxicity. (D) Groundwater quality will be monitored and soil remediation success evaluated. Untreated site (E) is used as a control for comparison of the remediation success. GW – groundwater; EK- electrokinetics.

# More information



#### For more information about the EiCLaR project visit **eiclar.eu** where you can find the following additional resources:

> Introductory video (EiCLaR in a nutshell) – a short video explaining the project eiclar.eu/category/videos/

- >Animated explainer video an animation aimed at a general audience to provide a broad understanding of the technologies within the EiCLaR project eiclar.eu/category/videos/
- Technical bulletins a series of short documents describing the EiCLaR outputs and signposting to more detailed information eiclar.eu/library/
- Podcasts interviews with some of the main people working on the EiCLaR project to find out a bit more about their backgrounds, how they got to their current positions and their role and activities within the project eiclar.eu/category/videos/
- > List of journal papers published in peer-reviewed scientific journals these outputs capture the most technical aspects of the research and are fundamental to demonstrate the quality of research being undertaken eiclar.eu/library/
- White papers a technical briefing note for each of the EiCLaR technologies, targeted to different practitioner audiences: for site owners/ managers (including real estate developers), for regulators, for service providers and for environmental service procurement personnel eiclar.eu/library/



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