



MIBIREM – Toolbox for Microbiome based Remediation

Deliverable 6.1

MIBIREM Report: How to conduct regulatory review for soil bioremediation

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D6.1 MIBIREM Report: How to conduct regulatory review for soil bioremediation

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1 Summary

In this deliverable, a detailed review of the EU and national regulatory situation on the application of bioremediation technologies is executed. With this information, we ensure that the MIBIREM pilots and TOOLBOX fulfil regulatory requirements. The focus of the review is on:

- The addition of bacteria / microbiomes to soil & groundwater
- The addition of supporting substances to soil & groundwater
- The 7 use-case countries: Austria, Belgium, France, Germany, Italy, Netherlands and Spain.

GMOs and pathogenic organisms that belong to Group III and IV are excluded from this review, because these are not part of the MIBIREM project.

Additionally, this review includes also a preliminary 'lessons learned' report on the MIBIREM use cases, informed by interviews with site owners / persons responsible of 7 contaminated sites across the EU. The main findings from these interviews highlight bioremediation as preferred alternative, stress the need for its broader adoption, underline the importance of site-specific approaches, and emphasise the key role of collaborative EU projects like MIBIREM in this regard.

EU-level

Regarding the environmental impact, there are no specific restrictions to the addition of Group I & II microorganisms and supporting substances. Most appropriate are more general rules on base of **Regulation (EU) 2019/1009** (Fertilizing products regulation), which is in fact limited to the upper meter of the soil:

- Micro-organisms can be used on/in soil, as long as they are non-GMOs and are not multiple resistant to antimicrobials
- Micro-organisms and supporting substances can be used on/in soil, as long as they are effective, properly stored and used for their intended purpose and do not present a risk to human, animal or plant health. In Annex I, limit values for contaminants (heavy metals, pathogens) in the products are mentioned, which must not be exceeded.

Regarding the handling of microorganisms in laboratories and in the field, both **Directive (EU) 2000/54/EC** (Risk related to exposure to biological agents at work) and **Technical guidelines on biological hazards in the working environment (UN ILO)** are in place and should be used.

National level

On national level, both German and Italian legislation are most restrictive for the use of Group I & II microorganisms and supporting substances in Soil & Groundwater remediation works. A site-specific impact and risk assessment regarding environment and human health is part of the approval procedure by the competent authorities. Microorganisms of Group II may be used for biotechnological applications under careful control and monitoring. In addition to potentially pathogens (Group II), in Germany this also involves microorganisms that produce 'toxins'. In Italy, specific attention must be paid to occupational safety, including training and worker protection for handling Group II agents.

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We recommend to connect to the most stringent regulations on either EU or national level in the 7 use case countries.

2 Introduction

2.1 Aim

One of the tasks within WP6 is to draw up a detailed review of the regulatory situation on the application of bioremediation technologies for use-case countries. The goal is to generate an overview of regulatory requirements on several levels (worldwide, EU as well as national) and consolidate this knowledge.

With this information, we ensure that the MIBIREM TOOLBOX – the microbiome and bioremediation tools developed and applied in MIBIREM - fulfils regulatory requirements and addresses market needs. This is important not only for our own use cases, but also for usability in future projects by ultimately improving customer access via a TOOLBOX environment (expected to be delivered at the end of the MIBIREM project).

2.2 Focus

The focus of the review of the regulatory situation on the application of bioremediation is on:

- The addition in the field of bacteria / microbiomes to soil & groundwater
- The addition in the field of supporting substances to soil & groundwater (substrates, nutrients, vitamins, electron acceptors / donors)
- The 7 use-case countries: Austria, Belgium, France, Germany, Italy, Netherlands and Spain.

2.3 Preconditions

Precondition for our project is that we do NOT work with and therefore do NOT focus on:

- Genetically modified microorganisms
- Pathogenic organisms that belong to Risk Group III and IV (see also paragraph 4.1.3).

3 Method

We used several sources to gather information on the regulatory situation:

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- Inventory executed by project GREENER (EU Horizon 2020)
- Publicly accessible literature of EU and national/regional environmental governments
- The practical experience of the MIBIREM-partners (consultancies / contractors / technology institutes) and their sister-companies.

In addition to this, we have already made an advance on the ‘lessons learned’ on use cases, by interviewing site owners / persons responsible (see Chapter 6). The full ‘Lessons Learned’ report will be delivered at the end of the project as part of D6.2 (Policy recommendations to stimulate uptake of soil bioremediation), when the pilot studies at some of the use cases have actually been executed.

4 Addition of bacteria / microbiomes to soil & groundwater

In this chapter, we focus on the regulatory with respect to addition of microorganisms (specifically bacteria) into soil & groundwater for remediation purposes. GMOs and pathogenic organisms that belong to Group III and IV are excluded from this review, because GMOs are not part of the MIBIREM project (we only work with bacteria that originate from soils & groundwater in the field). Group III and IV bacteria are excluded from enrichment cultures due to safety reasons in the laboratories of the MIBIREM-partners.

4.1 UN- and EU-regulatory provisions

4.1.1 Main literature resources

- [1] Regulatory, legal and risk assessment. GREENER (2020-2023), EU Horizon 2020. TAUW was project partner and co-authored this Deliverable
- [2] Regulation (EU) No 1143/2014 on the prevention and management of the introduction and spread of invasive alien species, 22 October 2014
- [3] DIRECTIVE 2000/54/EC on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC), 18 September 2000
- [4] Technical guidelines on biological hazards in the working environment, International Labour Organization (Meeting of Experts for the tripartite validation of the technical guidelines on biological hazards), Geneva, 20-24 June 2022
- [5] Regulation (EU) 2019/1009 on laying down rules on the making available on the market of EU fertilising products and amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009 and repealing Regulation (EC) No 2003/2003, 5 June 2019

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4.1.2 Environmental impact

The technologies in the MIBIREM project highly rely on the remediation action of existing microbial communities. This was also the case for the EU GREENER project (2019-2022), as part of which the legal framework on the use of microorganisms in the sector of remediation was screened [1]. No specific information could be identified. Given the lack of regulation in the use of bacteria or microorganisms in the wastewater treatment sector and waste management in general, they focused on the legislation for other sectors and activities using microorganisms for specific applications: the use of microorganisms in biofertilizers and biocontrol in agriculture.

Regulation 2017/1432 and 1107/2009

The following section gathers the legal texts applying to biocontrol in agriculture:

- Reg. 1107/2009 concerning the market uptake of plant protection products on the market and repealing Council Directives 79/117 / EEC and 91/414 / EEC. This regulation was amended by Reg. 2017/1432, which, among others, classifies microorganisms as active substances as mentioned in Reg. 1107/2009.

An important aspect provided by Regulation 2017/1432 is that **an active substance, which is a microorganism, may be considered as being of low risk, unless at strain level it has demonstrated multiple resistance to antimicrobials used in human or veterinary medicine.**

Regulation 2019/1009

Regarding the use of microorganisms in biofertilizers, Europe has been conscious of the interest of small and large companies in the business of products based on microorganisms, and has started creating a regulation to define rules for the availability of microorganism-based products on the market of what is called “**CE-marked fertilising products**” (Barquero et al. 2019).

Indeed, the European Parliament has launched a relatively new regulation, known as **Regulation (EU) 2019/1009**, to regulate the use of fertilizers and harmonize the market for production of these compounds. **This regulation** includes inorganic and organic fertilizers, and, for the first time, it **recognizes the use of microorganisms as biostimulants within the organic fertilizers.**

Most importantly, this new legislation shows the importance placed on the safety of the products used as fertilizers, because safety is mentioned in three points (53; 59; and 72). For example, point 53 says: “**EU fertilizing products should be placed on the market only if they are sufficiently effective and do not present a risk to human, animal or plant health, to safety or to the environment when properly stored and used for their intended purpose, or under conditions of use which can be reasonably foreseen, that is when such use could result from lawful and readily predictable human behaviour**”.

Note on soil fertilization amendments: Based on the regulation (EU) 2019/1009, 11 component material categories are defined and the requirements are described. One of them is CMC7: Microorganisms. Only a very limited list of micro-organisms may be used. These are the 4 genera:

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Azobacter spp., *Mycorrhizal fungi*, *Rhizobium spp.*, *Azospirillum spp.* **But note that several CMC (Compost (CMC3); Fresh crop digestate (CMC4); Digestate other than fresh crop digestate (CMC5); Food industry by-products (CMC6)) contain a high concentration and biodiversity of microorganisms that can be used no matter what.**

There is no specific international regulation applicable (yet) regarding the addition of microorganisms (non-GMOs) to contaminated soil and groundwater for remediation purposes.

The criteria mentioned in agricultural Regulation 2017/1432 and point 53 of agricultural Regulation (EU) 2019/1009 as mentioned in the above text could be used as generic guidance. In summary:

- Micro-organisms can be used on/in soil as long as they are non-GMOs, are not multiple resistant to antimicrobials and do not present a (direct) risk to health & safety or to the environment
- Micro-organisms can be used on/in soil as long as they are effective, properly stored and used for their intended purpose.

Regulation 2014/1143 [2]

To prevent the introduction and widespread of exotic and invasive species, including microorganisms, the Invasive Alien Species Regulation is in place.

Article 11 and Article 12 are about 'Invasive alien species of regional concern and species native to the (European) Union' and 'Invasive alien species of Member State concern'.

In the case of MIBIREM, all microbiomes originate from the EU, so native (indigenous) to the European Union. Then, Member States may establish a national list of invasive alien species of Member State concern. The Member States need to have informed the EU Commission and the other Member States about the species they consider to be invasive alien species of Member State concern and of the measures applied.

4.1.3 Human handling

Directive 2000/54/EC - Risk related to exposure to biological agents at work (September 2000)

[3]. Directive 2000/54/EC aims at the protection of workers against risks to their health and safety, including the prevention of such risks, arising or likely to arise from exposure to biological agents at work. This Directive applies to activities in which workers are (potentially) exposed to **biological agents** (micro-organisms, including those which have been genetically modified, cell cultures and human endoparasites, which may be able to provoke any infection, allergy or toxicity) as a result of their work. In such cases, the nature, degree and duration of workers' exposure must be determined to make it possible to assess any risk to the workers' health or safety, and to lay down the measures to be taken. In the case of activities involving exposure to several groups of biological agents, the risk shall be assessed on the basis of the danger presented by all hazardous biological agents present.

This assessment shall be conducted on the basis of all available information, including:

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- Classification of biological agents which are or may be a hazard to human health.
- Recommendations from a competent authority which indicate that the biological agent should be controlled in order to protect workers' health when workers are or may be exposed to such a biological agent as a result of their work.
- Information on diseases which may be contracted as a result of the work of the workers.
- Potential allergenic or toxigenic effects as a result of the work of the workers.
- Knowledge of a disease from which a worker is found to be suffering and which has a direct connection with his work.

Biological agents need to be classified into one of the four risks groups defined by the Laboratory Biosafety Manual of the WHO and the EU Directive 2000/54/EC, according to their level of risk of infection:

- Group I: Unlikely to cause human disease.
- Group II: It can cause human disease, but unlikely to spread to the community.
- Group III: It can cause severe human disease, and may present a risk of spreading to the community, but with effective treatment available.
- Group IV: It causes severe human disease, and may present a risk of spreading to the community without effective treatment available.

See also the below Table 1, adopted from the GREENER project [1].

Group	Description
Group 1 biological agent	• It is unlikely to cause human disease
Group 2 biological agent	• It can cause human disease and might be a hazard to workers. • It is unlikely to spread to the community. • There is usually effective prophylaxis or treatment available.
Group 3 biological agent	• It can cause severe human disease and present a serious hazard to workers. • It may present a risk of spreading to the community, but there is usually effective prophylaxis or treatment available.
Group 4 biological agent	• It causes severe human disease and is a serious hazard to workers. • It may present a high risk of spreading to the community. • There is usually no effective prophylaxis or treatment available.

Regarding the use of natural soil bacteria used for MIBIREM pilot studies and available according to the MIBIREM toolbox, the expected Risk Groups are limited to Group I and II.

If the biological agent to be assessed cannot be classified clearly in one of the groups defined in above Table, it must be classified in the highest risk group among the alternatives according to the directive. But a comment should be made here. From the Horizon 2020 project GREENER, we know that after checking the Annex III of Directive 2000/54/EC, none of the closest relative genome for those species which were identified at strain level in the GREENER project are listed in that Annex.

As it is stated under Annex III, several considerations must be kept into account:

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- Biological agents which have not been classified for inclusion in Groups II to IV of the list **are not implicitly classified in Group I**.
- For genera where more than one species is known to be pathogenic to man, the list will include those species which are known to be the **most frequently responsible for diseases**, together with a more general reference to the fact that other species of the same genus may affect health.
- When a whole genus is mentioned in the classified list of biological agents, it is implicit that the **species and strains known to be non-pathogenic are excluded**.

For the purpose of the assessment in the GREENER project, the following choices were made:

- The strains of the species which were only characterised until genera level will be considered to belong to biological agents' Group I.
- Those species that were characterised until strain level belong to biological agent Group I until they are included in Annex III list. **Currently, a list with all identified biological agents' Group II does not exist at European level.**

Annex V and Annex VI of Directive 2000/54/EC provide indications concerning containment measures and containment levels, and containment for industrial processes, respectively, **when workers are exposed to biological agents belonging to Groups II to IV**. These indications and recommendations are listed under Annex IX – Additional information related to Directive 2000/54/EC [risk related to exposure to biological agents at work] of this document.

Health risk management involves assessing the exposure of people to biological risk. No biological risk is considered when using microorganisms belonging to Group I. **For microorganisms belonging to Group II** (as well as for Group III and IV, which are not relevant for MIBIREM), **it is required to assess the exposure risks and the associated preventive measures**.

Among the obligations of employers when workers are potentially exposed to biological agents, the following points are stated in this Directive:

- **Replacement:** The employer shall avoid the use of a harmful biological agent if the nature of the activity so permits, by replacing it with a biological agent which, under its conditions of use, is not dangerous or is less dangerous to workers' health in the present state of knowledge.
- **Reduction of risks:** Where the results of the assessment reveal a risk to workers' health or safety, workers' exposure must be prevented. In case this is not technically feasible, the risk of exposure must be reduced to as low a level as necessary, to adequately protect the health and safety of the workers concerned.
- **Information for the competent authority, when requested, in terms of results of the risk assessment to workers' health and safety, accidents or incidents which may have resulted in the release of a biological agent and which could cause severe human**

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infection and/or illness, and the list of exposed workers to group 3 and/or group 4 biological agents as well as the medical record of the health surveillance of workers with biological agents (the last 2 documents must be kept for at least 10 years following the end of exposure).

- **Hygiene and individual protection:** Employers shall be obliged to take appropriate measures to ensure that:
 - Workers do not eat or drink in working areas.
 - Workers are provided with appropriate protective clothing.
 - Workers are provided with appropriate and adequate washing and toilet facilities.
 - Procedures are specified for taking, handling and processing samples of human or animal origin.
- **Information and training of workers:** Information includes potential risks to health, precautions to be taken to prevent exposure, hygiene requirements, wearing and use of protective equipment and clothing, and steps to be taken by workers in the case of incidents and to prevent them.
- **Notification to the competent authority:** Prior notification shall be made to the competent authority of the use for the first time of group II, III and IV biological agents. This notification shall include:
 - The name and the address of the undertaking and/or establishment
 - The name and capabilities of the person responsible for safety and health at work
 - The results of the carried-out assessment
 - The species of the biological agent
 - The protection and preventive measures that are envisaged.

Laboratories carrying out work which involves the handling of Group II, III or IV biological agents for research, development, teaching or diagnostic purposes shall determine the containment measures in accordance with Annex V of that Directive, to minimise the risk of infection.

The biological agents (microbiomes), which are isolated and enriched during the project, to be specifically applied in the different developed water and soil remediation technologies are deemed to be categorised according to the categories detailed in Directive 2000/54/EC.

Technical guidelines on biological hazards in the working environment (June 2022)

The International Labour Organization (UN ILO) recently published these technical guidelines [4]. It does not refer to the EU Directive 2000/54/EC [3] in any way, which is striking. Though this is not legislation, this document can be seen as a worldwide accepted guideline for health & safety regarding working with biohazardous materials. Since 1919, the ILO brings together governments, employers and workers of 187 Member States.

The guidelines are focused on prevention and control of work-related injuries, ill health, diseases, and dangerous occurrences and deaths related to exposure to biological hazards in the working environment. A biological hazard refers to any microorganism which can cause harm to human health. Health impacts could include infectious and non-infectious diseases and injuries.

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Within the scope of MIBIREM, specifically Chapter 2 is interesting: risk management at the workplace level. The management of biological hazards enables organizations to effectively identify the hazards and assess the biological risks inherent in their activities, and to develop prevention and mitigation strategies to eliminate, substitute, control, or reduce the risk so far as is reasonably practicable. Chapter 2 consists of three paragraphs:

- 2.1 Biological hazard identification and risk assessment
- 2.2 Control measures
- 2.3 Risk communication.

In paragraph 2.1 a non-exhaustive list of biological hazards associated with work activities is enclosed. The list of activities includes:

- Work in laboratory
- Work in refuse-disposal plants and sewage purification installations.

Though soil & groundwater remediation is not mentioned as activity, the work in sewage purification installations comes closest to it. The associated possible hazards and risks are:

- Infections and allergies caused by organic components of biowastes, including bacteria and their fragments, fungi and their spores and mycotoxins, viruses and prions, parasites and vector-borne diseases;
- Airborne transmission of viral bacteria and fungal pathogens and the substances and structures produced by them;
- Antimicrobial-resistant pathogens;
- Infections caused by wounds due to contact with contaminated sharp objects;
- Respiratory diseases such as tuberculosis, COVID-19 and influenza; and
- Direct contact with contaminated objects or persons.

NB: *The underlined text is not for MIBIREM purposes because we do not work with viruses, fungi, bacteria group III and IV pathogens or vectors.*

Hazard identification / risk assessment should consider the pathological mechanisms, modes of transmission (direct or indirect contact, aerosols, droplet spread, fomites, water, vectors, food, zoonotic) and routes of exposure (for example, inhalation, ingestion, dermal, percutaneous, mucous membranes, parenteral).

Regarding control measures, preventive and protective measures should be implemented in consultation with workers and their representatives, according to the following (summarized and tailored for MIBIREM purposes) principles and in line with the hierarchy of controls:

- (a) All premises used by workers, and the equipment of such premises, should be properly maintained and kept clean, and should have sufficient and suitable ventilation, natural or

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artificial or both. Sufficient and suitable washing facilities and sanitary conveniences shall be provided and properly maintained

- (b) Physical containment: provide protection by means of physical barriers that prevent the escape of biological agents from the containment area, including doors, waste-water management systems and so on
- (c) Work restrictions: keep as low as possible the number of workers exposed or likely to be exposed; limit the workload and restrict work to as few worksites as possible
- (d) Operational protection: minimize exposure by enforcing safe working techniques
- (e) Hazard minimization: carry out a combination of measures to reduce the consequences of exposure should it occur (for example, contingency plans, health and medical surveillance).

4.2 National / regional regulatory provisions

4.2.1 Environmental impact

For a detailed overview of the national soil related regulations of Austria, Belgium, France, Germany, Italy, Netherlands and Spain, please see Annex 1.

From this, and extensive experience of some of the MIBIREM partners with soil & groundwater remediation, the following general conclusions can be drawn.

4.2.1.1 Austria

Austrian regulations that deal with environmental protection and remediation, such as the act of remediation of contaminated sites (cf. newest document April 2024) or the water protection act (cf. newest document 2017) do not explicitly deal with microorganisms. Defined are only waste materials including Biogene substances such as organic waste (act of remediation of contaminated sites).

Safety of work is regulated by the Biological Agents Ordinance (VbA). This ordinance protects workers from hazards posed by biological agents, including microorganisms such as bacteria, viruses, fungi, and parasites. The VbA specifies how to handle these substances safely to minimize risks. There is no obstruction of use of Safety Group I or Safety Group II microorganisms in soil & groundwater bioremediation defined.

4.2.1.2 Belgium

Belgium knows three regions with their own regional government and regional legislation. In the following summary, we made this distinction as well.

Flemish legislation

- The use of genetic modified organisms or pathogens is according to VLAREM described in Category 51. The definition of pathogens in this case is: the set of human, phytopathogenic and zoo pathogenic agents of risk level II, III and IV. This means that **for Group II**

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microorganisms, also a sort of control program for biosafety infrastructure and containment measures needs to be submitted to the competent authority.

Brussels legislation

- The 'soil' legislation in Brussels does not include any specifications regarding the injection of microorganisms or substrates into the soil
- Brussels Environment has published several Codes of Good Practice. Three codes of practice cover treatments based on bioremediation:
 - Code of Good Practice N°4 – Stimulated attenuation of chlorinated solvents (CVOs) in soil under anaerobic conditions
 - Code of Good Practice N°7 - Biologically stimulated natural attenuation of the saturated zone by adding O₂ and nutrients
 - Code of Good Practice N°8 – Bioventing
- These Codes of Good Practice all have similar components. The addition of 'specific' microorganisms for the degradation of contaminants can be executed in the case that:
 - These (consortia of) microorganisms can be delivered by a laboratory that can guarantee their characteristics and their origin or by using samples from sites where a complete degradation has been shown
 - The degradation of contaminants in soil via naturally present microbes is too slow
 - The natural presence of specific microorganisms are <10³ to <10⁵ per gram soil
- In Chapter 10 of all Codes, the risks, policy and safety measures are mentioned regarding additives, but not related to microorganisms

Walloon legislation

- In short, for the Walloon region there are no apparent restrictions regarding the use of microorganisms in the context of a soil remediation
- There are only restrictions in place with regard to invasive exotic plants and animals.

4.2.1.3 France

There are no specific national regulations for soil protection (from an environmental point of view) regarding the use of non-GMOs in soil in France, other than a reference to Regulation (EU) 2019/1009, in which the use of microorganisms in biofertilizers and biocontrol in agriculture is described (see Chapter 4.1.2).

From a human health point of view, risk Group I and II (unless an exception is noted) are not listed as micro-organisms and toxins whose use could present a risk to public health (**Arrêté Du 26 Avril 2023 Fixant La Liste Des Micro-Organismes et Toxines Prévue à l'article L. 5139-1 Du Code de La Santé Publique** (Chapitre 9: Micro-organismes et toxines).

4.2.1.4 Germany

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In Germany, there are specific regulations and guidelines for the introduction of microorganisms into the subsurface, particularly with regard to environmental protection and public health. These provisions are part of the broader ‘Nationale Strategie zur Biologischen Vielfalt’ (NBS), which the Federal Government is implementing to conserve and sustainably use biological diversity in Germany.

The national regulations for the introduction of microorganisms into the subsoil in Germany are governed by various laws and ordinances (see Annex 1 for a more detailed description).

Several specific requirements and approval procedures must be observed when carrying out bioremediation in Germany. Here are the most important steps:

1. **Approval /permit requirement.** Most remediation measures require approval from the relevant authorities, such as the Federal Office for the Environment (BMU, Bundesamt für Umwelt) or the state environmental authorities. The introduction of microorganisms into the subsurface generally requires a permit from the competent authorities.
2. **Environmental impact assessment (EIA).** An EIA is required to assess the potential impact of the remediation measures on the environment.
3. **Risk assessment.** A comprehensive risk assessment must be carried out prior to approval to ensure that the microorganisms have no harmful effects on the environment or human health.
4. **Monitoring and control.** After authorization, regular monitoring and control measures are required to ensure that the remediation measures, including the microorganisms, are proceeding as planned and that no unexpected side effects occur.
5. **Approval procedures.** In accordance with the Bundesimmissionsschutzgesetz (BImSchG), approval procedures for facilities that could potentially harm the environment or human health.

‘Prohibited’ microorganisms are neither to be found in the “Biostoff-Verordnung” nor in the “Nationale Biodiversitätsstrategie”. However, there are clear distinctions in the use of microorganisms in biotechnology concerning their pathogenic potential. The “Biostoff-Verordnung” makes a strict distinction between microorganisms of risk Group 1, 2, 3 and 4 (categories based on EU regulations). There are almost no relevant restrictions for Group 1, but severe restrictions for Groups 3 and 4. Microorganisms of Group 2 (potentially pathogenic microorganisms) may be used for biotechnological applications under careful control and monitoring during handling and release. In addition to potentially pathogens, this also involves microorganisms that produce ‘toxins’, of which it is not sure whether this may be the case for intermediate metabolic products during degradation processes.

4.2.1.5 Italy

In Italy, the relevant regulations concerning the addition of microbiomes for soil and groundwater bioremediation are primarily derived from national legislations and European Union (EU) regulations. These include Legislative Decree No. 152/2006, Legislative Decree No. 81/2008, and

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various EU directives governing the use of biological agents in environmental and occupational health contexts. In general, the use of Group I and II bacteria is permitted for bioremediation, subject to specific regulatory conditions. Key aspects include:

- Group I and II bacteria can be used in soil and groundwater remediation projects provided they are non-GMO, non-pathogenic, and not listed as hazardous in Annex III of Directive 2000/54/EC.
- Prior approval from competent authorities, such as regional environmental agencies, is required under Legislative Decree No. 152/2006 for the addition of microorganisms. This involves risk assessments and containment measures tailored to the risk group of the microorganisms used.
- Specific attention must be paid to occupational safety under Legislative Decree No. 81/2008, which mandates detailed risk assessments, training, and worker protection for handling Group II agents.

4.2.1.6 Netherlands

In general, the relevant Dutch protection and planning acts and decrees regarding environment (incl. nature) and soil do not obstruct the use of Group I and II bacteria in soil & groundwater remediation projects. In other words, according to the Dutch regulation, bacteria can be added to soil for soil & groundwater remediation, as long as:

- It does not concern GMOs, bacteria of the coligroup, *Escherichia coli* or *Enterococcus*.
- There is good housekeeping in general, regarding cleaning up leaks and spills and using resistant tank or basins for the (microbial) substances it contains.
- Any person who deals with substances or mixtures, and who knows or could reasonably have suspected that his actions with that substance or mixture or organism may cause hazards to human health or to the environment, is obliged to take all measures that can reasonably be required of him in order to prevent or limit those hazards as far as possible.
Note: though this is specifically focused on chemicals and GMOs, it is reasonable to be valid for solutions with (non-genetically modified) bacterial microbiomes as well.

Though it might be expected regarding nature that it is not allowed to introduce (invasive) foreign (non-native) species, the Dutch Living Environment Activities Decree is restricted for this subject to animals and plants. Introduction of microorganisms (and fungi) is not regulated. This means that there are no restrictions (yet) regarding introduction of non-native microorganisms from the point of nature conservation.

4.2.1.7 Spain

The technical guide for the evaluation and prevention of risks related to exposure to biological agents (Royal Decree 664/1997, only available in Spanish) seems to be the most accurate regarding the specific purposes of MIBIREM. The Decree is a transposition of several European directives. This is a translated part from the introduction of the Spanish Decree:

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“...at European Union level, the relevant Directives have laid down general criteria for safety and health measures in the workplace, as well as specific criteria for measures to protect against accidents and risk situations. In particular, Directive 90/679/EEC of 26 November 1990 on the protection of workers from risks related to exposure to biological agents at work lays down specific minimum requirements in this field. This directive was subsequently amended by Directive 93/88/EEC of 12 October 1993 and adapted to technical progress by Directive 95/30/EC of 30 June 1995. This Royal Decree transposes the content of these three Directives into Spanish law”.

The technical guideline includes two types of situations regarding Health & Safety situations with biological agents:

1. Exposure arising from an occupational activity with deliberate intent to use or handle a biological agent, which is the main purpose of the work.
2. Exposure arising from an occupational activity that does not involve a deliberate intention to use or handle a biological agent but may lead to exposure.

Although it is indicated that soil remediation is included among the activities, there are no specific measures regarding H&S.

In fact, the content of the guideline from the International Labour Organization (see paragraph 4.1.3) seems to be similar to the Spanish guideline.

4.2.2 Human handling

In Chapter 4.2.1, we already covered some aspects related to the human handling of bacteria/microbiomes. Regarding human handling of Group I and II bacteria, the national (or regional) regulatory provisions are considered to be in line with or subordinate to the international regulatory provisions. For the international regulatory provisions, we refer to Chapter 4.1.3.

5 Addition of supporting substances to soil & groundwater

5.1 Supporting substances for biostimulation

Biostimulation for degradation of contaminants can take place by addition of supporting substances to soil and groundwater, either with or without the addition of microbiomes (bio-augmentation). For the addition of supporting substances, regulatory provisions might be in place which are also focused on environmental and human safety (potential exposure of these substances to workers).

The nutrients that are regularly used for biostimulation in soil are:

- Sodium nitrate (NaNO_3)
- Ammonium chloride (NH_4Cl)
- Sodium tripolyphosphate ($\text{Na}_5\text{P}_3\text{O}_{10}$)
- Ammonium phosphate ($(\text{NH}_4)_3\text{PO}_4$).

The substrates (carbon sources) that are regularly used for biostimulation in soil are:

- Molasses
- Protamylasse™
- Emulsified soya oil (or other vegetable based oil)
- Lactates (like sodium lactate, $\text{NaC}_3\text{H}_5\text{O}_3$)
- Acetates (like sodium acetate, $\text{NaC}_2\text{H}_3\text{O}_2$)
- Polylactate esters (food quality)
- Methanol.

Other substances for biostimulation in soil are:

- Vitamin B12 (catalyst for enzymatic processes)
- Calcium- or magnesiumperoxide (oxygen providers, ORC)
- Sodium bicarbonate (pH-buffer solution).

5.2 EU regulation

Regarding the addition of supporting substances to soil, the EU regulations focus specifically on the legislation for biofertilizers in agriculture to protect humans, plants, animals and the environment. In Chapter 4.1.2, we already described **Regulation (EU) 2019/1009** (Fertilizing products regulation) that regulates the use of fertilizers, soil improvers (incl. compost), growing media and plant biostimulants. This regulation includes inorganic and organic fertilizers, which may contain secondary raw materials or recovered waste, to which the before (cf. Chapter 5.1) mentioned nutrients and substrates can be put as well.

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This legislation shows the importance placed on the safety of the products used as fertilizers: “EU fertilizing products should be placed on the market only if they are sufficiently effective and do not present a risk to human, animal or plant health, to safety or to the environment when properly stored and used for their intended purpose, or under conditions of use which can be reasonably foreseen, that is when such use could result from lawful and readily predictable human behavior”.

However, this regulation is limited to products that are to be applied on plants or their rhizosphere or on the mycosphere, which in fact only covers the top soil (upper meter).

According to Regulation (EU) n° 2019/1009, an EU fertilising product shall:

- Meet the requirements set out in Annex I of that Regulation for the relevant product function category.
- Meet the requirements set out in Annex II of that Regulation for the relevant component material category or categories.

In Annex I of the EU Regulation, limit values for contaminants (heavy metals, pathogens) in fertilisers are mentioned which must not exceeded.

In the event of addressing any other aspect not covered by Annex I or II of the Regulation, EU fertilising products shall not present a risk to human, animal or plant health, to safety or to the environment.

Note: *Manufacturers have the possibility to choose between marketing their products in compliance with the Regulation or based on national rules alone, which implies the national rules can overrule the EU Regulatory ones.*

Regarding the use of additives in the deeper soil (>1 meter), which is used in most of the biostimulation projects, EU regulation is not present. In the case of deeper application, we suggest that the general precondition should be the same as for aspects that are not covered by Annex I or II of the Regulation: ‘supporting substances shall not present a risk to human, animal or plant health, to safety or to the environment’. This should be made plausible by some substantiation.

In a more indirect way, the European Water Framework Directive can also give rise to taking protective measures regarding the introduction of supporting substances to soil and groundwater, as far as these can eventually reach surface waters by drainage of groundwater.

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5.3 National/regional regulatory provisions

5.3.1 Austria

In Austria, the use of additives for in-situ remediation is decided by the respective representatives of the federal provinces, i.e. the provincial governors. Due to the existing laws, a distinction must be made here between remediation of the unsaturated zone (i.e. the soil) and the saturated zone (i.e. the groundwater). As groundwater is strictly protected in Austria, the authorities are stricter here, although the introduction of additives into contaminated areas, at least as far as contaminated sites are concerned, is now subject to the repair principle and not the precautionary principle, which makes no sense if the groundwater is already contaminated.

In the case of soil, the authorities usually take a much more relaxed view of the use of additives. Above all, there is no soil protection law in several federal states. And if there is, then it is usually aimed at agriculture and if so, then only marginally at contamination. There is no federal soil protection law in Austria. In future, the Europe-wide 'Soil Monitoring Law' would be relevant here - depending on whether or how quickly it is implemented.

The Federal Environment Agency and AGES have an advisory role.

5.3.2 Belgium

Flanders legislation

The VLAREM-legislation in Flanders (Decree on General Provisions on Environmental Policy (DABM)) states nothing specific regarding the injection of substrates in the subsoil regarding the supporting substances for biostimulation.

Brussels legislation

The 'soil' legislation in Brussels does not include any specifications regarding the injection of substrates into the soil.

Walloon legislation

It should be noted that in the Walloon Soil Decree, the definition of "pollutant" (article 2.2°) is quite broad and includes products, substances and chemical compounds, that, due to their concentration and generation by human activity, constitutes pollution (**Note:** *Soil pollution is defined as: 'Presence on or in the soil of pollutants that are harmful or may be harmful, directly or indirectly, to the quality of the soil'. Whereas there is no framework for non-standard chemicals to judge whether the presence in certain proportions is problematic or not).*

At the same time, we can conclude that there are no apparent restrictions regarding the use of substrates in the context of soil remediation.

5.3.3 France

Specific legislation for addition of supporting substances to soil & groundwater is not present in France.

According to the methodology for the management of polluted sites and soils (Code de l'environnement - Livre V : Prévention des pollutions, des risques et des nuisances (Articles L501-1 à L597-46)), any treatment that achieves the decontamination objectives and controls health risks, water resources and the environment can be implemented.

5.3.4 Germany

As already stated in Chapter 4.2.1.4, several specific requirements and approval procedures must be observed when carrying out bioremediation in Germany.

In addition to the general approval requirement of remediation measures by the relevant authorities, the following is required:

- An environmental impact assessment (EIA) is required to assess the potential impact of the remediation measures on the environment.
- A comprehensive risk assessment is required prior to approval to ensure that the supporting substances have no harmful effects on the environment or human health.
- In case of on-site facilities that could potentially harm the environment or human health an approval procedure is required in accordance with the Bundesimmissionsschutzgesetz (BImSchG).

5.3.5 Italy

In Italy, the review of the site remediation and risk management plan is carried out by a multi-agency review board led by the Provincial or Metropolitan City authority (or, for sites of national interest, the Ministry of Environment and Energy Security) with the involvement of Regional Environmental Protection Agency (ARPA) based on the risk-based procedure in Legislative Decree 152/2006.

The board would refuse any additive that is (i) a Persistent Organic Pollutant banned by Regulation (EU) 2019/1021, and (ii) a substance on REACH Annex XVII.

All other additives—including biodegradable surfactants, nutrient solutions, bulking agents and natural zeolites—may be approved if they pass the site-specific risk assessment.

5.3.6 Netherlands

On base of daily practice with the addition of supporting substances to soil & groundwater for biostimulation (as well as for chemical oxidation), the competent authorities have no specific demands in The Netherlands. Only on base of the monitoring results of soil and groundwater macroparameters during the inspection of the remediation result end situation, authorities can demand restoration of the natural situation. In general, this concerns pH, EC, T and sometimes phosphate, nitrate, sulphate and TOC/DOC.

In the Environment and Planning Act (Dutch: Omgevingswet), the **Living environment activities decree** (Dutch: Bal) is put in place. Chapter 2 of this decree is about environmentally harmful activities and discharge activities in general, of which Article 2.11 is about specific duty of care for environmentally harmful activities (Dutch: specifieke zorgplicht):

- When one can reasonably prevent environmental pollution, one should prevent this by any means.
- Good housekeeping, which is about the storage and handling before addition to the soil (e.g. cleaning up leaks and spills; having sufficient absorbents in stock; the resistance of a tank or basin to the substances it contains).

Specific legislation for addition of supporting substances to soil & groundwater is not present in the Netherlands.

5.3.7 Spain

On national (Royal Decree 665/2023) and regional level (Water Administrations, Confederaciones Hidrográficas), there are groundwater quality normatives (standard chemicals).

Remediation plans need to be approved, case by case, by the Administration after the assessment of remedial technologies proposed, including additives as substrate, nutrients, etc.

But there is no specific legislation on the addition of supporting substances to soil & groundwater in Spain.

6 Results on interview with site owners / persons responsible

6.1 Set-up and participants

Interviews were conducted with 7 site owners / persons responsible for remediation by using a questionnaire set up by RTDS in cooperation with MIBIREM WP6 partners. These are the site owners / persons responsible of the use cases that were sampled in WP1 for WP2 and WP3.

Note: only the PHC and cyanide use cases are covered in the interviews, not the HCH use cases due to various reasons – e.g. in the case of Colleferro, an interview was not possible with responsible authorities due to its sensitive status as a former military site. In the case of Bitterfeld, an interview

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could not occur due to a recent change in problem ownership of the site – another attempt will be made once the situation has been clarified at the site. The site owners at Sabiñánigo did not respond to the interview request.

The following questions were asked to site owners / persons responsible:

- Which industrial activities have used to occur on this site?
- What are the main sources of contamination, and what are the main contaminants?
- Is mainly soil or groundwater affected, or both?
- Are there people living near the site and if yes, which ones (e.g. farmers, citizens, etc)?
- Is there any pressure to remediate the site? From the public, authorities, economic actors or others?
- Have any remediation measures been undertaken on this site? If yes, which?
- In case remediation has not been started yet at your site: what is the reason for this?
- How familiar are you with bioremediation as a method for treating contaminated sites?
- Have you ever considered bioremediation as part of the solution at your site? Do you think that bioremediation could benefit your site? If yes, how?
- What would keep you from bioremediation or soil remediation in general?
- MIBIREM is working on microbiome-based bioremediation. For an explanation of this technology we refer to our website [Mibirem.eu](https://mibirem.eu) and our [animation video](#). What do you think about the potential of this approach to remediate your site?
- **For potential pilot sites only** - MIBIREM is planning to start a pilot on your site next year. What do you expect from this pilot for your site?
- What do you think of current regulations (e.g. local, national, EU level) on remediation of contaminated sites? Do current regulations stimulate you in finding a solution and are they enabling a swift and efficient remediation process? Or does regulation pose an obstacle and if yes, in which ways?
- Do you feel the need for more support of environmental consultants / engineers and academia in convincing the authorities on the benefits and feasibility of bioremediation?
- Any concluding remarks?

6.2 Results

The contamination of soil and groundwater at various sites, primarily due to industrial activities such as gas production, oil recycling, and manufacturing, has resulted in widespread pollutants like PAHs, hydrocarbons, heavy metals, and cyanides (see Figure 1 on the following page). The proximity of residents and businesses to many contaminated areas highlights the urgency of remediation, especially in urban settings like Amersfoort and Rotterdam.

6.2.1 Remediation measures across sites

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- Amersfoort and Rotterdam sites (Kralingen & Feijenoord): Legal obligations drive rapid soil remediation to facilitate urban development, while authorities focus on balancing spread and degradation of contaminants at contamination boundaries.
- Stockach and OVAM Lumco sites: Remediation efforts are constrained by financial factors and uncertainties in results. Stockach stands out for its groundwater focus and reliance on subsidies to manage costs.
- Kralingen and Feijenoord sites: These sites have transitioned to monitoring natural attenuation with adapted groundwater models, benefiting from supportive Dutch regulations that prioritize effective remediation solutions.
- Rinteln and Ploufragan sites: Rinteln emphasizes eco-friendliness and operational continuity, despite challenges in estimating remediation costs and timelines. Ploufragan advocates for bioremediation's financial and environmental benefits but faces hurdles due to limited funding and regulatory metrics.

In Annex 2 of the Deliverable, a summary can be found of the Pollution Levels at the Sites and the Corresponding Remediation Measures.

Where is the contamination?

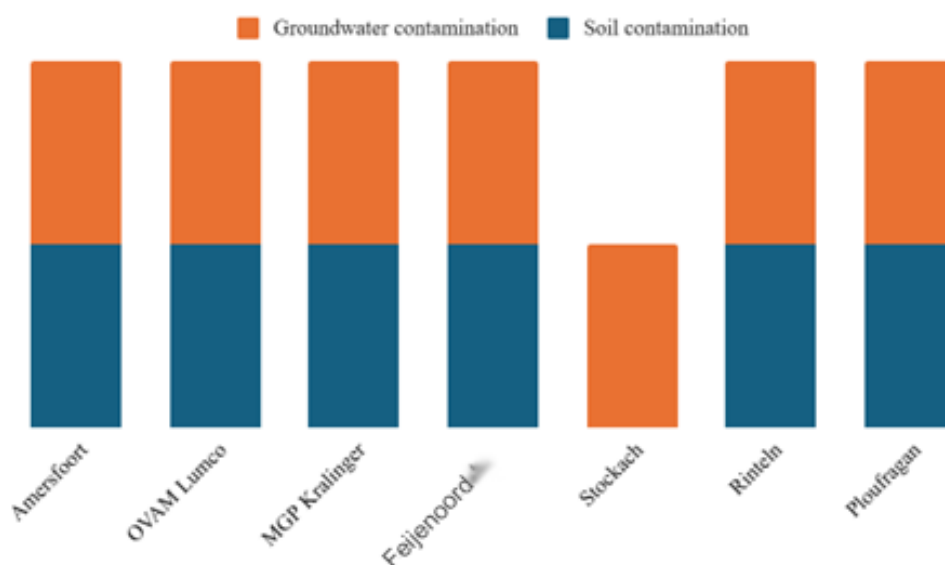


Figure 1. Presence of contaminants at each site

6.2.2 Bioremediation Insights and Key Observations

Bioremediation, including the MIBIREM approach, is recognized as an eco-friendly and efficient method for addressing contamination. While promising, it faces challenges such as financial constraints, site-specific conditions, and regulatory complexities. Key observations include:

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1. Benefits of Bioremediation: Sites value its ability to naturally degrade contaminants, adapt to specific conditions, and reduce environmental impact.
2. Barriers: Financial limitations, site accessibility, and complex regulations frequently hinder implementation. Solid technical foundations are crucial for acceptance, as emphasized in Dutch cases.
3. Expectations for MIBIREM: Many sites express optimism about the approach's potential to improve remediation outcomes and contribute to sustainability (Figure 2).

Familiarity with Bioremediation

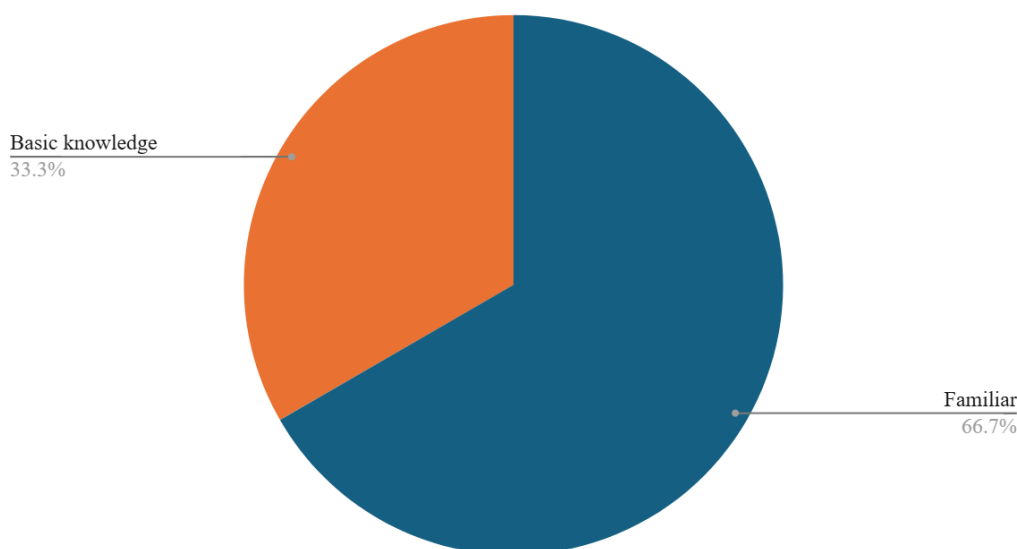


Figure 2. Site owners' familiarity with bioremediation techniques.

6.2.3 Regulatory Challenges and Recommendations

Regulatory perspectives differ across sites. While Dutch regulations are seen as supportive, other regions face complexities or lack of incentives. Recommendations voiced in the interviews by site owners / persons responsible include:

- Sustainability Obligations: Advocating for legal frameworks to enforce sustainable remediation practices.
- Research and Site Models: Continued studies and detailed models are critical for overcoming uncertainties and fostering public and authority support.
- Funding and Zoning Improvements: Addressing financial barriers and expediting planning processes can significantly advance remediation efforts.

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In Annex 3 of the Deliverable, a summary can be found of the answers to the questions regarding Bioremediation Acceptance and Regulatory Issues.

7 Conclusions and Recommendations

7.1 Regulatory provisions on addition of micro-organisms to Soil & Groundwater

EU regulation on adding micro-organisms to soil & groundwater

- The legal framework on EU level on the addition of Group I and Group II micro-organisms to soil & groundwater for bioremediation was screened and no specific information could be identified.
- **Regulation (EU) 2019/1009** - Fertilizing products regulation. This regulation for agriculture recognizes the use of microorganisms as biostimulants within the organic fertilizers. From this, the following can be derived:
 - Micro-organisms can be used on/in soil, as long as they are non-GMOs, are not multiple resistant to antimicrobials and do not present a (direct) risk to health & safety or to the environment.
 - Micro-organisms can be used on/in soil, as long as they are effective, properly stored and used for their intended purpose.
- **Regulation (EU) 2014/1143** - Invasive Alien Species Regulation. This regulation is in place to prevent the introduction and widespread of exotic and invasive species, including microorganisms. Article 11 is about '*Invasive alien species of regional concern and species native to the (European) Union*'. In the case of MIBIREM, all microbiomes originate from the EU, so native (indigenous) in this sense. Article 12 is about '*Invasive alien species of Member State concern*'. For this, Member States should establish a national list of invasive alien species of Member State concern and publish it to the European Commission and the other Member States. We have not traced such a list for bacteria species.

EU regulation on human handling of micro-organisms

Human handling involves work in laboratory as well as in the field (like sewage purification installations but also soil & groundwater remediation works).

- **Directive (EU) 2000/54/EC - Risk related to exposure to biological agents at work (September 2000)**. This Directive aims at the protection of workers that are (potentially) exposed to **biological agents** (micro-organisms) against risks to their health and safety. Biological agents need to be classified into one of the four risks groups defined by the Laboratory Biosafety Manual of the WHO and the EU Directive 2000/54/EC, according to their level of risk of infection:
 - Group I: Unlikely to cause human disease.
 - Group II: It can cause human disease, but unlikely to spread to the community.

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- Group III and IV: It can cause severe human disease and may present a risk of spreading to the community, with (III) or without (IV) effective treatment available. The list with these groups is not complete and in some cases customisation (research on pathogenicity) is needed. The directive includes risk assessment and containment measures as part of the health risk management. For microorganisms belonging to Group II (as well as for Group III and IV, which are not relevant for MIBIREM), it is required to assess the exposure risks and the associated preventive measures.
- **Technical guidelines on biological hazards in the working environment, UN ILO (June 2022).** The International Labour Organization (UN ILO) published these technical guidelines. It does not refer to the EU Directive 2000/54/EC in any way, which is striking. Though this is not legislation, the document can be considered as a worldwide (i.e. the ILO has 187 Member States) accepted guideline for health & safety regarding working with biohazardous materials. Within the scope of MIBIREM, specifically Chapter 2 is interesting: risk management at the workplace level.

National regulations on adding micro-organisms to soil & groundwater

For Austria, Belgium, France, Germany, Italy, Netherlands and Spain, national regulations were examined.

In **Austria, France and Spain** there are no specific national regulations for soil protection from an environmental point of view regarding the use of non-GMOs.

In **Belgium** the Flemish legislation describes the use of genetic modified organisms or pathogens according to VLAREM in Category 51, which includes Group II microorganisms (used in MIBIREM in addition to Group I microorganisms). This means that a sort of control program for biosafety infrastructure and containment measures needs to be submitted to the competent authority.

In Brussels and Wallonia, there is no legislation on this issue. There are a few instructions included in the Codes of Good Practice published by Brussels Environment for addition of 'specific' microorganisms:

- Either guaranteed microorganisms by a laboratory or inoculants from sites where degradation has been shown.
- Addition in case the natural degradation of contaminants in soil is too slow.
- Addition in case the natural presence of specific microorganisms is $<10^3$ to $<10^5$ per gram.

In **Germany**, there are specific regulations and guidelines for the introduction of microorganisms into the subsurface with regard to environmental protection, governed by various laws and ordinances. Most important steps when carrying out bioremediation are:

1. The introduction of microorganisms into the subsurface requires a permit from competent authorities.
2. An environmental impact assessment is required to assess the potential impact.
3. A risk assessment must be carried out regarding environment or human health.

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4. Monitoring should ensure that microorganisms are proceeding as planned.
5. An approval procedure for facilities that could harm the environment or human health.

Microorganisms of Group II may be used for biotechnological applications under careful control and monitoring. In addition to potentially pathogens (Group II), this also involves microorganisms that produce toxins.

In **Italy**, in general the use of Group I and II bacteria is permitted for bioremediation, but prior approval from competent authorities is required under Legislative Decree No. 152/2006 for the addition of microorganisms, which involves risk assessment and containment measures tailored to the risk group of the microorganisms used.

In the **Netherlands**, Group I and II bacteria can be added to soil for soil & groundwater remediation, as long as:

- It does not concern bacteria of the coligroup, *Escherichia coli* or *Enterococcus*.
- There is good housekeeping in operation, containment and cleaning up leaks and spills.

7.2 Regulatory provisions on addition of supporting substances to Soil & Groundwater

Biostimulation for degradation of contaminants can take place by addition of supporting substances to soil and groundwater, either with or without the addition of microbiomes (bio-augmentation). It concerns:

- Nutrients (N, P)
- Substrates (C)
- Miscellaneous substances (vitamins, oxygen providers, pH-buffer).

EU regulation on adding supporting substances to soil & groundwater

Most applicable is **Regulation (EU) 2019/1009** (Fertilizing products regulation), that regulates the use of fertilizers, soil improvers (incl. compost), growing media and plant biostimulants. This regulation covers inorganic and organic fertilizers, to which nutrients and substrates can be put as well. EU fertilizing products should be placed on the market only if they are sufficiently effective and do not present a risk to human, animal or plant health, to safety or to the environment when properly stored and used for their intended purpose. In Annex I of the Regulation, limit values for contaminants (heavy metals, pathogens) in fertilisers are mentioned which must not be exceeded. However, this regulation is limited to the topsoil (upper meter) and any national rules can overrule this EU regulatory provisions.

In a more indirect way, the European Water Framework Directive can also give rise to taking protective measures as far as applied supporting substances can eventually reach surface waters.

National regulations on adding supporting substances to soil & groundwater

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In **Austria**, the use of additives for in-situ remediation is decided by the respective representatives of the federal provinces, i.e. the provincial governors. For groundwater (saturated zone), it is stricter. There is a precautionary principle to protect groundwater quality, but in case of contaminated areas, the 'repair principle' can overrule this.

In **Belgium**, the Flanders and Brussels legislation do not state specifics regarding the injection of supporting substances for biostimulation. In Wallonia, the definition of "pollutant" (article 2.2°) is quite broad and can include products, substances and chemical compounds. At the same time, we can conclude that there are no apparent restrictions regarding the use of substrates as far as this is not harmful to the quality of the soil.

In **France**, specific legislation for addition of supporting substances to soil & groundwater is not present, and any treatment that achieves the decontamination objectives and controls health risks, water resources and the environment can be implemented.

In **Germany**, in addition to the general approval requirement of remediation measures by the relevant authorities, an environmental impact assessment (EIA) is required as well as a comprehensive risk assessment regarding effects on the environment or human health (same as for using microorganisms). For on-site facilities, an approval procedure is required.

In **Italy**, the site remediation and risk management plan are reviewed by the competent authorities. Only Persistent Organic Pollutants and substances on REACH Annex XVII are refused. Other additives need to pass the site-specific risk assessment.

In **the Netherlands**, it is quite similar to France. In general, environmental pollution should be prevented by any means and good housekeeping is key. In practice, only if macroparameters during monitoring after active remediation give rise to questions on impact of the natural situation, authorities demand restoration.

Although remediation plans need to be approved, including additives, there is no specific legislation on the addition of supporting substances to soil & groundwater in **Spain**.

Results on interviews with site owners / persons responsible

Based on the analysis provided in Chapter 6, the following key findings emerge:

- **Bioremediation as a Preferred Alternative:** Bioremediation is gaining acceptance as an effective and environmentally sustainable alternative to traditional methods, particularly in areas like the Netherlands, where regulatory frameworks support its use.
- **Importance of Site-Specific Approaches:** Tailored remediation approaches, guided by solid technical foundations and comprehensive site models, are pivotal for success.
- **Need for Broader Adoption:** To expand bioremediation's benefits, regions should streamline regulations, provide financial incentives, and prioritize sustainability-focused decision-making.

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- Role of Collaboration and Research: Strong partnerships with remediation experts and continued research into microbial processes and site-specific dynamics are essential for addressing uncertainties and optimizing outcomes.

7.3 Recommendations

For the MIBIREM pilots as well as for the MIBIREM TOOLBOX, we recommend connecting to the most stringent regulations on either EU or national level in the 7 use case countries to have a uniform approach. This means the use of:

- Regulation (EU) 2019/1009 (Fertilizing products regulation) on addition of micro-organisms and additives to Soil & Groundwater. **We suggest that the intention of this Regulation should be the same for deeper application of supporting substances, and not only be limited to the top soil (upper meter).**
- Directive (EU) 2000/54/EC (Risk related to exposure to biological agents at work) on handling of micro-organisms.
- Technical guidelines on biological hazards in the working environment (UN ILO) on handling of micro-organisms.
- German and Italian legislation that both demand a site-specific impact and risk assessment regarding environment and human health on handling and addition of micro-organisms and additives.

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Annex 1: Overview of national regulations per country

Regulations in Austria

- Altlastensanierungsgesetz (currently updated and should be entering into force in 2025) - no mentioning of microbes: [RIS - Altlastensanierungsgesetz - Bundesrecht konsolidiert, Fassung vom 30.04.2024 \(bka.gv.at\)](#)
 - [BGBLA 2024 I 30.pdfsig \(bka.gv.at\)](#)
- Wasserrechtsgesetz (Water rights act): [RIS - Wasserrechtsgesetz 1959 - Bundesrecht konsolidiert, Fassung vom 25.04.2017 \(bka.gv.at\)](#)
- Federal soil protection
 - On state level, deals however not in all cases with contaminated sites
- Environmental law (Umweltrecht)
- Verordnung der Bundesministerin für Arbeit, Gesundheit und Soziales über den Schutz der Arbeitnehmer/innen gegen Gefährdung durch biologische Arbeitsstoffe

Regulations in Belgium

Flemish legislation

- The VLAREM-legislation in Flanders (implementing decree of the Decree on General Provisions on Environmental Policy (DABM)) states nothing specific regarding the injection of microorganisms or substrates in the subsoil, except that the injection of (dangerous) products in the groundwater is prohibited or strictly regulated (Category 52: Indirect discharge in groundwater)
- The injection of non-dangerous products (no substances from the list of Appendix 2B of VLAREM) is regarded as an artificial supplement of the groundwater (Category 54 of VLAREM). No specific injection volume or rate is mentioned, but based on the description in Article 2.54, this Category 54 seems only to be applicable for higher injection volumes or rates
- The use of genetic modified organisms or pathogens is according to VLAREM described in the Category 51: Use of genetic modified organisms or pathogens. The definition of pathogens in this case is: the set of human, phytopathogenic and zoo pathogenic agents of risk level II, III and IV. This means that for Group II micro-organisms also a sort of control program for biosafety infrastructure and containment measures needs to be submitted to the competent authority. If this is the case in practice in Flanders is unknown.

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OVAM published three 'Codes of Good Practice' (CoGP) with regard to application of bioremediation:

- Code of Good Practice In Situ Bioremediation of Chlorinated Solvents (2007)
- Code of Good Practice Natural Attenuation (2003)
- Code of Good Practice In Situ Bioremediation Petroleum Hydrocarbons (2005).

In these CoGP the legislation with respect to the application of fungi, bacteria and other microorganisms is not discussed.

In a fourth publication by OVAM, "Code of Good Practice Phytoremediation" some legislation is mentioned in the guideline, but this is more about the treatment of biomass-waste, protection of endemic species, forestation and deforestation, etc. In other words, no mentions of restrictions regarding the use of certain plant species or microorganisms.

Brussels legislation

- The 'soil' legislation in Brussels does not include any specifications regarding the injection of microorganisms or substrates into the soil.

Brussels Environment has published 10 Codes of Good Practice + 3 auxiliary Codes of Good practice on treatment topics. Three codes of practice cover treatments based on bioremediation:

- Code of Good Practice N°4 – Stimulated attenuation of chlorinated solvents (CVOs) in soil under anaerobic conditions. These (consortia of) microorganisms can be delivered by a laboratory that can guarantee their characteristics and their origin or can be sampled at sites where a complete dechlorination has been shown (paragraph 3.5). In Chapter 10 risks, policy and safety measures are mentioned regarding additives, but not related to microorganisms
- Code of Good Practice N°7 - Biologically stimulated natural attenuation of the saturated zone by adding O₂ and nutrients. The addition of 'specific' microorganisms for the degradation of contaminants can be executed in the case that (Paragraph 6.2):
 - The degradation of contaminants in soil via naturally present microbial flora is too slow, even after using additives (O₂, nutrients, ...)
 - The microorganisms needed for natural degradation are not or insufficiently present in soil (<10⁵ per gram)
 - Laboratory tests show the additional value of microorganisms from generic and specific cultures in case of the absence of biodegradation potential at the site.

In Chapter 10 risks, policy and safety measures are mentioned regarding additives, but not related to microorganisms

- Code of Good Practice N°8 – Bioventing. Also in this Code of Good Practice, though focussed on the use of 'indigenous' microorganisms, the addition of specific microorganisms is suggested. In this case, an insufficiency of naturally present population of <10³ per gram is mentioned. Chapter 10 is similar to Code of Good Practice N°4 and N°7

Walloon legislation

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In short, we can conclude that for the Walloon region there are no apparent restrictions regarding the use of microorganisms, substrates, bacteria in the context of a soil remediation.

Nothing specific either for the use of plants in the scope of phytoremediation, but one has to take into consideration the following information related to invasive species:

- Circular on invasive exotic plants of May 30, 2013 (Circulaire relative aux plantes exotiques envahissantes du 30 mai 2013) (openjustice.be)
- Decree (dated 2/05/2019) on the prevention and management of the introduction and spread of invasive exotic species (+ a few orders dated 2002 : [Législation | Invasives | La biodiversité en Wallonie](#))
- Website on invasive exotic species : [Les espèces exotiques envahissantes | La biodiversité en Wallonie](#) (by the way, as you can imagine, the listed invasive species focus on plants and animals, not on (micro) organisms)

Regulations in France

- Code de l'environnement - Livre V : Prévention des pollutions, des risques et des nuisances (Articles L501-1 à L597-46)
 - Most important law regarding environment and pollution management
 - No specific regulations about introduction of non-OGM micro-organisms (for soil and groundwater remediation)
 - The national policy for the management of polluted sites and soils is a risk management policy taking into account the specific usage of the site. According to this methodology, any treatment that achieves the decontamination objectives and controls health risks, water resources and the environment can be implemented.
- Health risk management
 - Code du travail – Chapitre 1 : Dispositions générales
 - Arrêté Du 16 Novembre 2021 Fixant La Liste Des Agents Biologiques Pathogènes : Health risk management involves assessing the exposure of workers to biological risk. In the labor code, biological agents are classified into four groups based on the significance and risk of infection:
 - No biological risk is considered when using microorganism belonging to group 1. For microorganisms belonging to group 2, it is required to assess the exposure risks and the associated preventive measures. The use of group 3 or 4 microorganisms does not seem practical for the implementation of depollution treatments given the infection risks.
- Code de la santé publique - Chapitre 9 : Micro-organismes et toxines. (Articles L5139-1 à L5139-3)
- Arrêté Du 26 Avril 2023 Fixant La Liste Des Micro-Organismes et Toxines Prévues à l'article L. 5139-1 Du Code de La Santé Publique : This chapter covers micro-organisms and toxins whose use could present a risk to public health, as well as products containing them.

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- The classification in the labor code is used. Group I and II (unless an exception is noted) are not listed as micro-organisms and toxins whose use could present a risk to public health
- Environmental risk management : Regulation (EU) 2019/1009 Rules on the Making Available on the Market of EU Fertilising Products :
 - Based on the regulation (EU) 2019/1009 , the fertilizing products are available on the market if there is no significant risk to human, animal or plant health, to safety or to the environment. It may be easier to use authorized products commonly spread on soils as fertilizing products than to apply solutions that are rarely used, having a similar function (stimulating the biological activity of soil) and requiring to argue on risk management.
 - 7 product function categories are defined, and the requirements are described : Fertilizer , Liming material, Soil improver, Growing medium, Inhibitor, Plant biostimulant, Fertilising product blend
 - 11 component material categories (CMC) are defined, and the requirements are described :
 - Virgin material substances and mixtures, Plants, plant parts or plant extracts, Compost, Fresh crop digestate, Digestate other than fresh crop digestate, Food industry by-products
 - Micro-organisms. Only a very limited list of micro-organisms may be used. These are the 4 genera: Azobacter spp., Mycorrhizal fungi, Rhizobium spp., Azospirillum spp. Note that several CMC (compost, digestate, food industry by-product) containing a high concentration and biodiversity of micro-organisms can be used.
 - Nutrient polymers, Polymers other than nutrient polymers...

Regulations in Germany

- Federal nature conservation act (BNatSchG)
 - Protection of nature and landscape
- Federal soil protection act (BBodSchG)
 - §4 duties to prevent danger
 - §11-16 Supplementary regulations for contaminated sites
- Federal soil protection and contaminated sites ordinance (BBodSchV)
 - §10-17 Investigation, assessment and remediation of harmful soil changes and contaminated sites
- Environmental damage act (USchadG)
 - § 8 Determination of remediation actions

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- Water resources act (WHG)
 - §48 keeping the groundwater clean
 - §90 remediation of groundwater damages
- Gene technology act (GenTG)
 - only concerns the handling, release of genetically modified organisms
- Ordinance on biological substances (BioStoffV)
 - for activities involving hazardous biological substances, protection of persons
 - §6 Activities without protection level assignment
 - (1) Activities that do not fall under Section 5 (1) do not have to be assigned to a protection level. These are activities within the meaning of Section 2(7)(2). These activities include, for example cleaning and remediation work [...]

In Germany, there are specific regulations and guidelines for the introduction of microorganisms into the subsurface, particularly regarding environmental protection and public health.

These provisions (Permit requirement; Risk assessment; Monitoring and control; Prohibited microorganisms) are part of the broader Nationale Strategie zur Biologischen Vielfalt (NBS), which the Federal Government is implementing to conserve and sustainably use biological diversity in Germany.

The national regulations for the introduction of microorganisms into the subsoil in Germany are governed by various laws and ordinances. Here are some of the most important ones:

- 1. Gesetz über die Umweltverträglichkeitsprüfung (UVPG): This act regulates the environmental impact assessment for certain projects, including the introduction of microorganisms into the subsurface.
- 2. Bundesnaturschutzgesetz (BNatSchG): This act protects nature and the environment and contains provisions on the approval of measures that may affect the environment.
- 3. Trinkwasserverordnung (TrinkwV): This ordinance specifies the requirements for the quality of drinking water and contains regulations for the prevention of contamination by microorganisms.
- 4. Biodiversity and agriculture (Biodiversity Act): This act promotes the conservation of biodiversity and contains provisions on the sustainable use of microorganisms.

There are certain microorganisms that may not be introduced into the subsoil for bioremediation; whereas there are almost no relevant restrictions for Group 1, but severe restrictions for classes 3 and 4. These restrictions are based on the Biostoffverordnung (BioStoffV) and other relevant laws and regulations. Here are some important points:

- 1. Hazardous / pathogenic microorganisms: microorganisms classified as biological agents of risk Group 3 or 4

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- 2. Toxic microorganisms: microorganisms that produce toxins (unsure, whether this is the case for intermediate products during degradation processes).

Several specific requirements and approval procedures must be observed when carrying out bioremediation in Germany. Here are the most important steps:

- 1. Approval requirement: most remediation measures require approval from the relevant authorities, such as the Federal Office for the Environment (BMU) or the state environmental authorities.
- 2. Environmental impact assessment (EIA): An EIA is required to assess the potential impact of the remediation measures on the environment. This includes the analysis of the planned measures and their possible negative consequences¹.
- 3. Risk assessment: A comprehensive risk assessment must be carried out prior to approval to ensure that the microorganisms do not have any harmful effects on the environment or human health
- 4. Monitoring and control: After authorization, regular monitoring and control measures are required to ensure that the remediation measures are proceeding as planned and that no unexpected side effects occur.
- 5. Approval procedures in accordance with the Bundesimmissionsschutzgesetz (BImSchG). This law regulates the approval procedures for facilities that could potentially harm the environment or human health.

These procedures ensure that bioremediation is carried out in a safe and environmentally friendly manner. It is important to observe the specific requirements and approval procedures of the competent authorities.

- Sources: www.bmu.de ; www.bgbau.de ; www.weka.de

DGUV = Instructions for action for risk assessment according to the Ordinance on Biological Substances

- 4.1.2 Liquid media for groundwater or soil remediation
 - The use of laboratory or starter cultures and improper handling of nutrient solutions and handling of nutrient solutions and with recirculated groundwater can lead to the increased occurrence or multiplication of biological agents of risk group 2. When growing specialty cultures, there is a particular risk of the proliferation of the bacterial genera *Acinetobacter*, *Pseudomonas* and *Nocardia*. Representatives of these genera have a good degradation potential.

Regulations in Italy

- Legislative Decree No. 152/2006 - Environmental Code
 - Cornerstone of Italy's environmental regulatory framework.
 - Addresses soil remediation by setting criteria for identifying contaminated sites and outlines the responsibilities and procedures for remediation.
 - For groundwater, it stipulates measures to prevent contamination and procedures to remediate contaminated groundwater.

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- Includes extensive provisions on water treatment and waste management, establishing standards for waste disposal, recycling, and recovery, and setting guidelines for wastewater treatment and discharge.
 - The decree does not explicitly focus on the use of microbiomes. It allows for the use of any effective and safe remediation technology, which includes bioremediation methods.
 - Chemical amendments must be approved and monitored to ensure they do not pose additional environmental risks. The implementation of any remediation technique must comply with national safety and environmental standards, and detailed impact assessments and monitoring are required to ensure the protection of human health and the environment.
- EU Regulation 1107/2009 – Plant protection products
 - Governs the authorization and use of plant protection products (PPPs) in the EU, with a strong emphasis on safety for human health and the environment. 75/2010
 - Chemicals, plant extracts, and microorganisms acting against pests or influencing plant growth must be approved based on their efficacy, safety for human health, and minimal environmental impact.
 - Certain active substances may be identified as candidates for substitution if they pose greater risks to human health or the environment.
 - Mandates thorough assessment and approval processes for microorganisms used as active substances in PPPs, ensuring they do not adversely affect human health or the environment.
 - The use of genetically modified organisms (GMOs) in plant protection products is also subject to specific controls and must meet additional safety criteria.
 - The regulation sets strict limits on contaminants in PPPs to minimize environmental and health risks.
 - EU Regulation 1009/2019 (as effective from 2022) - Fertilising products
 - Applies to a broad range of fertilizing products including organic and organo-mineral fertilizers, soil improvers, inhibitors, and plant biostimulants.
 - Introduces strict safety and quality requirements for fertilizing products, including maximum permissible levels of contaminants and pathogens.
 - Lists specific component materials that can be used in EU fertilizing products.
 - By setting limits on toxic contaminants and promoting the use of recycled materials, the regulation aims to protect the environment and support the EU's circular economy objectives.
 - Pathogens referred to include:
 - Salmonella spp.
 - Escherichia coli
 - Listeria monocytogenes

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- *Vibrio* spp.
- *Shigella* spp.
- *Staphylococcus aureus*
- Enterococcaceae

Regulations in the Netherlands

- Wet milieubeheer (Wm) – Environmental protection act ([wetten.nl - Regeling - Wet milieubeheer - BWBR0003245 \(overheid.nl\)](#))
- Wet bodembescherming (Wbb) – Soil protection act ([wetten.nl - Regeling - Wet bodembescherming - BWBR0003994 \(overheid.nl\)](#))
- Omgevingswet – Environment and Planning act ([wetten.nl - Regeling - Omgevingswet - BWBR0037885 \(overheid.nl\)](#))
 - Besluit activiteiten leefomgeving (Bal) - living environment activities decree ([wetten.nl - Regeling - Besluit activiteiten leefomgeving - BWBR0041330 \(overheid.nl\)](#))
 - Besluit bodemkwaliteit – Soil quality decree (<https://wetten.overheid.nl/BWBR0022929/2024-01-01>)
- Wet Natuurbescherming - Nature conservation act = fully replaced by Environment and Planning act
- Beoordelingsrichtlijnen (BRL) 6000 and 7000 – Assessment directives (quality) for soil remediation
- Water framework directive EU = incorporated into ‘Besluit kwaliteit leefomgeving’ – Environmental quality decree ([wetten.nl - Regeling - Besluit kwaliteit leefomgeving - BWBR0041313 \(overheid.nl\)](#))
- Environment and Planning Act (Omgevingswet)
 - Most important law regarding environment, nature and water, active since January 1st 2024
 - No specific regulations about introduction of bacteria (for soil and groundwater remediation) (non-GMOs)
 - Living environment activities decree (Bal), Chapter 2 (environmentally harmful activities and discharge activities: general), Article 2.11 = specific duty of care for environmentally harmful activities (specifieke zorgplicht)
 - When one can reasonably prevent environmental pollution, one should prevent this by any means
 - Good housekeeping (e.g. cleaning up leaks and spills; having sufficient absorbents in stock; the resistance of a tank or basin to the substances it contains)
 - Living environment activities decree (Bal), Chapter 3 (environmentally harmful activities and discharge activities: direction indicator), Paragraph 3.2.23 = Remediation of the soil as environmentally harmful activity

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- Only for soil remediation, not for groundwater or waterbed remediation
 - Limited to rearrangement, excavation and removal of soil (Bal, Paragraph 4.121)
- Living environment activities decree (Bal), Chapter 11 (activities concerning nature), Paragraph 11.2.10 = Activities that (may) result in the introduction or spread of invasive alien species
 - Animals and plants are mentioned, micro-organisms are not; though the Dutch Senate asked for it in 2020 ('Foreign/non-native species: species, subspecies or lower taxa of animals, plants, fungi or micro-organisms introduced outside their natural range'), which means that there are no restrictions (yet) regarding introduction of (non-native) micro-organisms from the point of nature conservation
- Environmental protection act (Wet milieubeheer)
 - Largely replaced by Environment and Planning act, but the following points are still regulated through the Environmental protection act: substances, waste, (greenhouse gas emissions), public access to environmental information and environmental liability (<https://iplo.nl/regelgeving/wet-milieubeheer/>)
 - Substances and products: Chapter 9 of Environmental protection act
 - Article 9.2.1.2: 'Any person who professionally manufactures, imports, applies, processes, treats or makes available to another person a substance, mixture or genetically modified organism in the Netherlands, and who knows or could reasonably have suspected that his actions with that substance or mixture or organism may cause hazards to human health or to the environment, is obliged to take all measures that can reasonably be required of him in order to prevent or limit those hazards as far as possible'.
Note: This article is limited to chemical substances, blends of them and GMOs
- Environmental quality decree
 - Appendix 5 belonging to Article 2.15: Environmental threshold values for water abstracted at a water abstraction site for the preparation of water intended for human consumption
 - Only restrictions for bacteria of the coligroup, Escherichia coli, and Enterococcus

Regulations in Spain

- Soil quality national normative (Royal Decree 9/2005)

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- Soil quality normative, including investigation, assessment criteria, risk assessment & remediation of contaminated sites.
- Soil quality regional normative (Comunidades Autónomas)
 - Soil quality criteria for metals: established by Regional Authorities due to the spatial difference in natural concentrations of metals.
 - Some Regional Authorities had established soil regional normative (Decrees): Pais Vasco, Cataluña, Andalucía, etc.
 - Technical guidelines in some regions: Madrid, Pais Vasco, Cataluña, Andalucía, etc.
- Groundwater quality national normative (Royal Decree 665/2023)
 - Groundwater quality normative, including investigation, assessment criteria, risk assessment & remediation of contaminated sites.
- Groundwater quality regional normative (Water Administrations, Confederaciones Hidrográficas)
 - Regional normative, regional criteria, technical guidelines.
- Groundwater quality
 - keeping the groundwater clean
 - remediation of groundwater punctual damages
 - case by case decision: Remediation Plan to be approved by the Administration, after the assessment of the remedial technologies proposed (including consortium and additives as substrate, nutrients, etc.).
- Technical guide for the evaluation and prevention of risks related to exposure to biological agents. [Royal Decree 664/1997] & Order of 25 March 1998.
 - H&S risk related to: *“Exposure arising from an occupational activity with the deliberate intent to use or handle a biological agent, which is the primary purpose of the work. That is, the cultivation, handling or concentration of biological agents whether at industrial or experimental levels, or for research, commercial or therapeutic purposes”*. Soil remediation activities are specifically included.

Annex 2: Summary Pollution Levels and Remediation Measures from interviews with site owners / responsible persons

Site name	Role	Industrial activities at the site	Contamination sources and type	Soil, groundwater, or both?	Nearby residents	Pressure to remediate	Remediation measures undertaken	Reasons for no remediation
Amersfoort	Municipality of Amersfoort is responsible for clean-up	Oil and tar (byproducts of gas production)	Tar, PAHs, and mineral oils	Both	Yes, living near and on the site	Development projects require rapid soil remediation to prepare the subsurface for construction.	Explosives removed, soil excavated, partial construction completed, groundwater extracted, and pure product removed	-
OVAM Lumco	Neither site owner nor responsible for clean-up, only test site	Lamp black production, roofing felt production, gas production, cast asphalt installation, industrial expansion (varnishes, mastics, polyurethane), operation of combustion ovens.	PAH, mineral oil, BTEX, heavy metals	Both	Yes, living 50 m away	Partial implementation for site redevelopment into an SME zone	Partial excavation completed; in-situ remediation ongoing with dual-phase extraction	-
MGP Kralingen	City of Rotterdam is the site owner and responsible for clean-up	Gas plant	PAHs, cyanides, VOHs, hydrocarbons	Both	Yes, urban site	Remediation in the 1990s: soil excavation and groundwater treatment due to public health concerns and legal	Deep soil excavations and ~20 years of groundwater pump-and-treat with biological methods.	-

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						obligations		
Feijenoord	City of Rotterdam is the site owner and responsible for clean-up	Creosole plant (wood preserving) and municipal gaswork	PAHs, cyanides VOHs mineral oil	Both	Nursery home in the northern part of the site	Legal obligation to control groundwater contamination; apartment building with underground parking planned near nursery home.	Polluted soil excavated; groundwater bioremediated	Zoning plan for the apartment building pending; northern site remediation awaits nursing home decommissioning.
Stockach	Neither site owner nor responsible for clean-up	Production of town gas (coking plant)	Coal and Cyanide	Mainly groundwater	Yes, citizens and small businesses	No pressure	Remediation begins in spring 2025	Start in 2025
Rinteln	Neither site owner nor responsible for clean-up	Town gas production (precursor to natural gas) and disposal of production waste	Cyanide and PAHs	Both	Yes, private homes	Proactive remediation monitored by local soil and water authorities ; public informed and engaged.	Apart from the ongoing remediation, no further ones are known	-
Ploufragan	Neither site owner but responsible for contaminated abandoned sites	Oil recycling factory, Industrial soap production, paints and solvents	Hydrocarbons, PAHs, Phenols, COHV	Both	Yes, residents	No pressure, the industry is no longer operating, municipality not interested in the site	Tanks and visual waste removed in 2000; building deconstructed in 2017.	Studies ongoing; remediation costs up to 5M€, with 1.5M€ available.

Annex 3: Summary Bioremediation Acceptance & Regulatory from interviews with site owners / responsible persons

Site name	Familiarity with bioremediation	Bioremediation benefits	Barrier/enablers to implement bioremediation	MIBIREM approach - acceptance	Expectations from the MIBIREM strategies at pilot site	Opinions on regulation	Need for more support	Concluding remarks
Amersfoort	Familiar	Groundwater contaminants degrade biologically, balancing spread and degradation at contamination boundaries.	Approach must align with project, timeline, finances, and development opportunities	They seem promising and may contribute to my project in the future	Not a pilot site	Regulations are complex, involving many parties, but ensure measurable and protective measures	Understanding the possibilities and assessing success chances beforehand is key to effective project planning.	None
OVAM Lumco	Familiar	Bioremediation, like stimulated natural attenuation, may address residual pollution post-in-situ phase.	Uncertainty of results	See the answers to the two questions before	Not a pilot site	Traditional techniques are invasive and environmentally impactful, leaving room for less harmful alternatives	None	None
MGP Kralinger	Familiar	Groundwater treatment ended in 2023; new phase monitors Natural Attenuation and assesses risks with site-specific groundwater models	n/a	Groundwater and soil provide anaerobic conditions with adapted bacteria, enabling effective bioremediation	Not a pilot site	Dutch regulations encourage and support effective remediation solutions	Bioremediation is now well accepted in NL, provided authorities receive a solid technical foundation for its implementation.	None

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Feijenoord	Quite familiar	Bioremediation applied in the south; limited contaminant spread elsewhere due to poor soil permeability and bacterial degradation.	The availability and accessibility of the site	Spontaneous processes in Rotterdam soil likely limit contaminant spread more than expected.	Not a pilot site	Dutch regulations encourage and support effective remediation solutions	Bioremediation is now well accepted in the Netherlands, provided authorities receive a solid technical foundation for its implementation.	Further research into the complex processes by micro-organisms in the soil remains very important
Stockach	Sufficiently familiar, as I was involved in the test trial.	Remediation starts in 2025	The costs, but since there are subsidies, I am relaxed about it	n/a	Quick remediation needed to maintain storage use, with no alternative space available	Disapproves due to increased costs being passed to citizens	none	None
Rinteln	Only basic knowledge has been acquired through the project at our site (since 2021). The initiative originated from AG Mull & Partner / Sensatec.	Yes, the decision was made accordingly at our site.	The remediation time is harder to estimate compared to conventional remediation, and so are costs. Achieving the remediation target value, as with conventional methods, is also not guaranteed.	Chosen remediation method allows operations to continue, avoids demolition, is eco-friendly, and cost-effective	Not a pilot site	Decision-making involved limited regulatory interaction, as Mull & Partner managed communication. Remediation was deemed essential for public welfare	We received strong support from our remediation partners, convinced authorities of the method, and advocate for a legal sustainability obligation.	The restoration project, launched in 2021, shows promising progress despite initial risks from its unprecedented scale and approach.
Ploufragan	Limited soil characterization knowledge personally; colleague specializes in	Bioremediation offers financial and carbon impact reduction compared to thermic desorption. The	Financial and carbon-free benefits, along with improved soil health	Lots of hopes for this technology	To assess the feasibility of this technology for addressing complex and severe	French regulations prioritize remediation efficiency	No idea	Site models are essential for demonstration purpose

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	bioremediation research	site benefits from ample remediation time, despite challenges with used-oil and COHV contamination. Research encourages continued studies.			contamination	y over technology-based metrics like carbon efficiency, consumable efficiency, or soil health		s and maintaining good publicity among authorities
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