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/ Report

PHYTOREMEDIATION

Code of Good Practice

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This code of good practice deals with the remediation of contaminated soils and groundwater through phytoremediation. The code contains a general description of the technology (literature study) and specific guidelines for the design, maintenance and monitoring of remediation by means of phytotechnology.

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1 INTRODUCTION

Phytoremediation is a sustainable, green remediation technology that uses plants and their associated microorganisms to remediate contaminated soil, (ground)water or sediment via degradation, extraction, stabilization, transformation or volatilization. Phytoremediation is an important innovative, sustainable and low cost remediation alternative compared to traditional remediation techniques. Additional benefits are also expected in terms of the quality of the living environment, natural capital, biodiversity and ecosystem services.

In Flanders, phytoremediation is used in a limited extend. A possible reason for this is the lack of practical experience and/or technical knowledge. This document provides answers and tools for each of these limitations.

1.1 PURPOSE

This report serves as a guide for policy makers and soil remediation experts in the evaluation of phytoremediation as a relevant technical option for the remediation of contaminated soils, surface and groundwater and sediments. This document also provides an overview of current knowledge regarding plant based technologies and provides procedures that help in the evaluation of phytoremediation possibilities. The principles and guidelines that are described are valid for contaminated industrial sites, private sites, residential areas, nature reserves, parks, etc. Phytoremediation can be applied to a wide variety of sites provided that a number of preconditions are met, as described in this document.

The sub-objectives are:
- Provide a description of the different phytoremediation mechanisms;
- Provide an overview of common types of contaminants and phytoremediation applications;
- Provide a description of environmental factors that can be used by soil remediation experts in the field when deciding and applying phytoremediation for a specific site. This also includes a critical evaluation of plant species;
- Provide a description of the added value of phytoremediation with regard to sustainability, quality of life and biodiversity;
- Establish a procedure for feasibility testing of phytoremediation;
- Prepare a procedure for the design, implementation, maintenance and monitoring of phytoremediation during the remediation works;
- Provide a procedure for closing strategy and aftercare for phytoremediation;
- Provide an overview of safety aspects;
- Give practical examples that illustrate possible phytoremediation applications.

1.2 PREPARATION OF THIS DOCUMENT

The following approach was used to compile the information on phytoremediation processes and procedures:
- An extensive literature study was conducted;
- Researchers and institutions were involved (Centre for Environmental Sciences (UHasselt), bio2clean, Arcadis and Witteveen+Bos) to obtain information about phytoremediation applications and costs;
- Current research projects and field applications of phytoremediation were evaluated. An overview of phytoremediation technologies that have already been implemented in practice was drawn up.

All gathered information was used for the preparation of feasibility, implementation, monitoring and aftercare procedures.

1.3 WHY CHOOSE A NATURE BASED REMEDIATION TECHNIQUE?

Institutions such as OVAM play a key role in restoring and maintaining the natural capital, biodiversity and the ecosystem services. The remediation of contaminated soils, sediment and water is aimed at restoring the disturbed ecosystems and restoring ecosystem functions, such as water treatment and the formation of raw materials and building materials.

This is possible on the one hand with the traditional remediation technologies such as pump & treat with aboveground water treatment systems, excavation and off sit treatment of contaminated soil, ‘in situ’ treatment by means of chemical oxidation and thermal techniques. For many contaminated sites, however, less invasive, nature based remediation technologies such as phytoremediation, are available. Such nature based technologies can complement the traditional remediation or be used a stand-alone technology. Phytoremediation includes all plant based technologies for the remediation of contamination. This includes planting of greenery, planting of deep-rooted trees to pump contaminated groundwater (mineral oil and volatile organic compounds) and/or to degrade contaminants in the root system, and creating buffer zones along fields to prevent leaching of fertilizers and pesticides into surrounding watersources. In addition to the traditional remediation technologies, phytoremediation plays an important role in maintaining or increasing biodiversity, but also in managing nature to restore ecosystem functions such as water treatment and the formation of raw materials and building materials. It also contributes to the creation of green living and working environment and offers educational and aesthetic benefits that improve the overall quality of the living environment and our natural capital.

Phytoremediation has many advantages over traditional remediation techniques. Since plants play the leading role, phytoremediation works largely on solar energy. It also has minimal operation and maintenance cost, the remediation takes place on site and no transport is required. In addition, phytoremediation also results in a reduced infiltration of rainwater, resulting in a decreased leaching of the contamination into the groundwater.

1.4 CONTENT AND OBJECTIVES

1.4.1 Literature study

A literature study with the state of the art knowledge (chapter 2) was conducted:
- the different mechanisms and processes regarding phytoremediation;
- the types of contaminants;
- the plant species;
- the different terrain characteristics that impact the technology and the added value in terms of sustainability, quality of life and biodiversity.

1.4.2 Procedures

The following procedures for phytoremediation are reported:
- Procedure for feasibility studies and pre design (Chapter 3);
- Procedure for design, installation, management and monitoring (Chapter 4);
- Procedure for closing strategy and aftercare (Chapter 5).

1.4.3 Safety and health aspects

Chapter 6 deals with the specific safety and health aspects of phytoremediation.

1.5 GLOSSARY

Allelopathy	A process by which plants, algae, bacteria and fungi produces substances that influence the growth of other organisms.
Apoplast	The apoplast in a plant is the space where passive, non-selective diffusion of substances takes place. It includes the cell walls and intercellular spaces.
Assimilates	Assimilates are substances that are produced in the leaves by sunlight and that are mainly transported through the phloem.
Bioaugmentation	Process in which specially selected microorganisms (bacteria, fungi) are added to the contaminated soil to degrade the contaminant and also often to improve plant growth.
Biochar	A solid material obtained by thermochemical conversion of biomass in a low-oxygen environment (pyrolysis). Definition from the International Biochar Initiative, 2012.
Conjugation	Conjugation occurs in bacteria and leads to the transfer of DNA from one cell to another cell connected via a pilus.
Endophytes	Endophytic microorganisms live in plants and are not pathogenic to the plant but support or promote the plant growth.

Evapotranspiration	A sum of evaporation (movement of water to the atmosphere from the ground, foliage) and transpiration through vegetation.
Exudate	Exudates (in the context of plants) are low and high molecular weight components (sugars, amino acids, peptides) that are excreted by the plant (for example through the roots).
Exudation	Process in which exudates are released, amongst other ways, via the plant roots.
Phloem	Phloem is the living tissue in vascular plants that transports water and assimilate. Phloem is composed of sieve vessels and associated cell.
Photo-autotrophic	Able to build up own organic molecules from inorganic molecules using sunlight.
Phreatophyte	Plant that roots down to the groundwater table.
Phytodegradation	Process in which plants and their associated microorganisms take up contaminants and degrade contaminants in plant tissues through metabolic processes or enzymatic activity.
Phytoextraction	Process in which plants and their associated microorganisms take up and store contaminants in plant tissue.
Phytohydraulics	Process in which plants and their microorganisms take up and evaporate water and thereby influence the groundwater level, the direction and velocity of the groundwater flow.
Phytoremediation	A remediation technology that uses plants and microorganisms to remediate contaminated soil, water or sediment via degradation, extraction, transformation or volatilization or to stabilize (immobilization) the contamination.

Phytostabilisation	Process in which plants and their microorganisms stabilize and/or immobilize certain contaminants in the soil, rhizosphere or in the roots.
Phytovolatilization	Process in which plants and their microorganisms take up, transport and volatize contaminants through transpiration.
Hydrophobicity	Having little or no affinity for water, water-repellent.
Hypha	The long, branching filamentous structure of a fungus.
Inoculation	The introduction of microorganisms into the soil.
Log K_{ow}	Octanol/water partition coefficient, the ratio of the concentration of the chemical in the octanol phase to its concentration in the water phase of a two-phase octanol/water system.
Metalloids	The group of semi-metals with properties that can be situated between the metals and non-metals.
Microorganisms	Organisms with cell dimensions < 0.2 mm that can be single or multi-cell. Examples are bacteria (Eubacteria and archaea), cyanobacteria (Eubacteria), moulds and fungi (Eukaryotes), protozoa (Eukaryota) and algae (Eukaryota).
Microbe-assisted phytotechnology	Specially selected microorganisms are introduced into the soil to accelerate the degradation of organic contaminants, to improve the extraction or stabilization and to promote plant growth.

Microbiome	All of the microorganisms in a certain environment.
Mutualism	Mutualism is an interaction between two life forms in which both benefit from that interaction.
Plasmid	A circular strand of DNA that is outside of the chromosomal DNA of bacteria.
Rhizodegradation	Process in which contaminants in the rhizosphere are degraded by a combined action of enzymes released from plants and microorganisms in the rhizosphere.
Rhizodeposition	All of the cells, mucous layers, inorganic components and exudates that plants can release through the roots.
Rhizofiltration	A form of phytoremediation in which water moves through the roots and in which toxic substances are removed.
Rhizosphere	The thin layer of soil around plant roots that is under the influence of the plant and their associated microorganisms and where the microorganisms exert a strong influence on the plant.
Sequestration	Storing/depositing, or keeping separate in, for example, plant tissue.
Symbiosis	The long-term coexistence of two or more organisms of different species, whereby the co-existence offers benefits for both organisms.
Transgenic plants	Plants in which one or more genes of another species have been introduced into the genome by genetic engineering.

Wicker cultivation	Growing willow trees/twigs; dense willow plantations on wet soils. The wood is cut down after one or a few years and used for basket weaving, bank retaining elements, tying up plants, etc.
Xylem	The wood vessels and associated elements in the guide tissue of vascular plants that is responsible for the transportation of water and nutrients from the roots to the leaves.

1.6 LIST OF ABBREVIATIONS

BTEX	Benzene, Toluene, Ethylbenzene, Xylenes
°C	Celsius degrees (temperature)
DDE	dichlorodiphenyl dichloroethane
DNT	dinitrotoluene
DNA	deoxyribonucleic acid
m	Metre (distance)
m-bgl	Metre below ground level (depth)
MTBE	Methyl tert-butyl ether
K	hydraulic conductivity
KEGG - database	Kyoto Encyclopaedia of Genes and Genomes database
K_{ow}	Octanol water partition coefficient
SRF	Short Rotation Forestry
PAHs	Polycyclic aromatic hydrocarbons
PPE	Personal protective equipment
PCBs	Polychlorinated biphenyls
PCE	tetrachloroethylene
POP	persistent organic contaminant
TCE	Trichloroethylene
TNT	Trinitrotoluene
TPH	Total Petroleum Hydrocarbons (mineral oil)
VOC	Volatile organic components
µg/l	Microgram per litre (concentration)
2,4-D	2,4-dichlorophenoxy acetic acid

2 LITERATURE REVIEW

This chapter explains the concept of phytoremediation, gives a brief overview of the mechanisms and processes related to phytoremediation, the types of contaminants, the environmental factors that influences the technology, the plant species and finally the added value in terms of sustainability, quality of life and biodiversity.

The aim is to provide the soil remediation expert with information on the mechanisms and applications of phytoremediation and to provide them with a tool to determine whether phytoremediation will be successful at a specific site.

2.1 DEFINITION

Phytoremediation includes a range of technologies that use plants and their associated microorganisms to capture, remove, convert and degrade contaminants in the soil, (ground)water and sediment. Phytoremediation differs from other biological remediation techniques because it uses living microorganisms in collaboration with living plants to remove or stabilize contaminants from the environment. In recent years, instead of the traditional term “phytoremediation”, the term “phytotechnologies” has often been used to emphasize that it also includes plant based technologies that stabilize contaminants. This is due to the fact that the term phyto “remediation” is often misinterpreted as plant based remediation techniques with the sole aim of “removing” the contaminants.

Some phytoremediation applications can be used as a main remediation technology (whether or not in combination with other remediation techniques) for the remediation of contaminated soils, groundwater and sediment, while others can be used as aftercare after applying traditional remediation methods (e.g. after excavation). It is also possible to use phytoremediation as an additional soil remediation technique, for example in combination with an excavation or floating layer removal, in which phytoremediation is then used to remove the residual contamination or to remove the contamination plume.

Phytoremediation can be used:

(1) for the remediation of moderate, low or high concentrations of inorganic and organic contaminants, even if they are spread over large areas;
(2) for the remediation of remaining contamination after removal of source zones with traditional remediation (e.g. excavation, multi-phase extraction)
(3) to prevent the infiltration of contaminants into groundwater or to reduce the leaching of fertilizers and pesticides into rivers,
(4) to control the spreading of diffuse contaminants (e.g., air deposition) and
(5) to provide an active form of controlled natural attenuation.

Phytoremediation can remove or stabilize a wide variety of contaminants including metals and organic contaminants such as volatile water soluble components, polycyclic hydrocarbons, mineral oil and explosive residues, as explained in the following sections.

It should be noted that the knowledge of phytoremediation is evolving continuously and very quickly. Certain guidelines will need to be periodically reviewed and updated based on the latest information, knowledge and insights.

2.2 MECHANISMS AND PROCESSES

Phytoremediation is a broad concept. The removal or stabilization of contaminants in soil, sediment, or groundwater and surface water by phytoremediation can be done through various mechanisms and processes. These mechanisms are related to the processes that plants use to take up organic and inorganic contaminants, but the role of the plant-associated microorganisms is also of great importance, which is discussed in more detail below in Section 2.2.1.

In **"standard" phytoremediation**, plants and their naturally occurring associated microorganisms are used. The variety of plant microorganism symbioses, contaminants and contaminated media (soil, (ground) water, sediment) that can occur lead to a number of different (classical) phytotechnology mechanisms that can be applied: phytodegradation, rhizodegradation, phytovolatilization, phytostabilization, phytoextraction and phytohydraulics (2.2.2).

However, if limitations occur with "standard" phytoremediation, **microorganism assisted phytoremediation** may offer a solution (2.2.3). A selection of microorganisms is enriched in the rhizosphere and/or inside the plant. For clarification, a comparison is then made with other, better known, remediation techniques such as bioremediation, natural attenuation and "pump and treat" (2.2.4); and some phytotechnology applications are explained in practice (2.2.5).

2.2.1 General

To determine which phytoremediation mechanism can be applied, it is crucial to know whether the contamination can be taken up by the plant and/or is biodegradable.

Uptake of the contamination

The uptake of the contaminant mainly takes place via the roots, subsequently transported to the aboveground parts for accumulation or degradation (Figure 1). The uptake of organic substances is strongly dependent on the hydrophobicity of the molecules, as well as on the selected plant species and the environmental conditions. Hydrophobicity is expressed as the log K_{ow} (logarithm of the octanol water partition coefficient). In general, a log K_{ow} of 0.5 - 3.5 means good uptake by plants, while substances with a higher log K_{ow} value will mainly adsorb to plant roots with no or very little translocation to the aboveground parts. Very water soluble contaminants, on the other hand, penetrate the xylem vessels fairly quickly before they can be degraded by microorganisms in the rhizosphere. Endophytes (the bacteria and fungi that live in the plant) play a crucial role in the degradation of this type of contaminants. This is discussed later.

After uptake by the plant, the uptake of the substances can go different ways including: phytodegradation, rhizodegradation, phytovolatilization and phytoextraction.

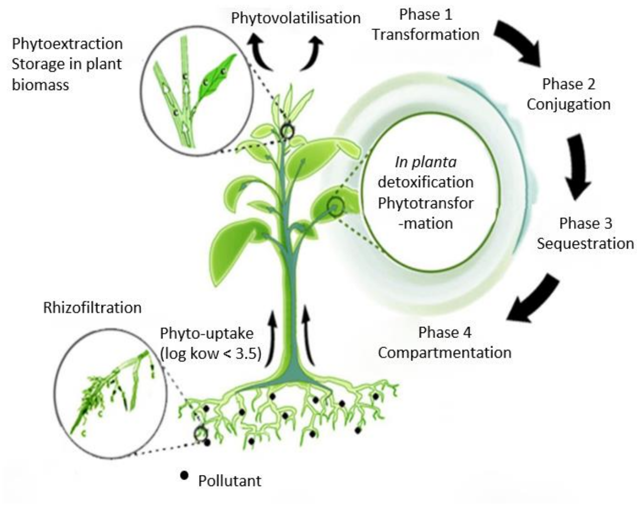


Figure 1: Plant uptake, transformation and degradation of contaminants in the plant. (green liver model) Adapted from Van Aken et al. (2009).

Degradation of the contamination

Plants are as photoautotrophic organisms not evolutionarily equipped with enzymes to metabolize organic substances and contaminants (compared to heterotrophic organisms such as animals and humans). Plants will therefore not degrade substances, but rather transform them into more water soluble and less harmful forms according to the so-called green liver model (**Figure 1**). Contaminants without a reactive group first enter phase 1 and are activated by redox reactions (e.g. a functional group is put on the molecule such as hydroxyl, amino or sulfhydryl). In phase 2 these substances are conjugated to sugars by e.g. glutathione and UDP-glycosyl transferases; ultimately, they are sequestered, usually in the vacuole or cell wall and finally stored in less photosynthetically active tissues including old leaves, in the roots, or in the woody material of the plant.

In addition to transformation by the plant itself, there are the plant associated microorganisms, the microbiome, which can collectively catalyse complete degradation of organic substances into CO_2 and water due to their wide variety of metabolic enzymes (**Figure 2**).

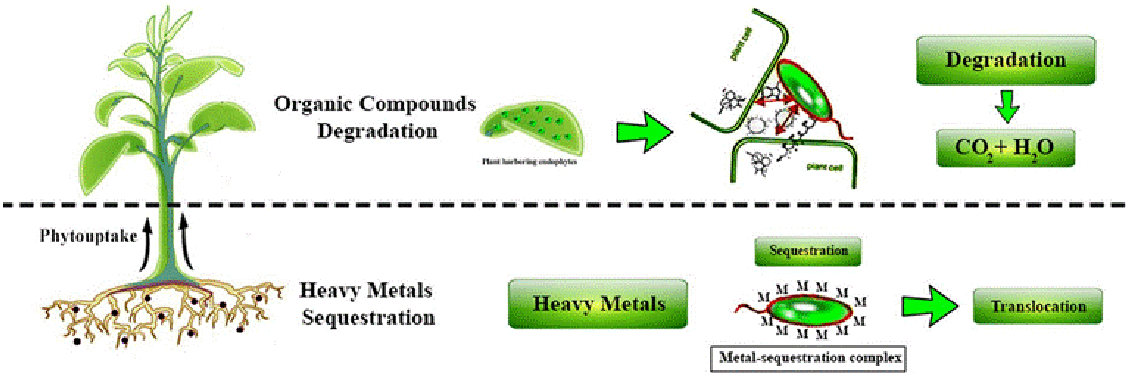


Figure 2: Endophytes in action against organic and inorganic contaminants (Weyens et al. 2009)

No matter which phytoremediation mechanism is applied, the **role of the plant associated microorganisms** is undeniable. Plants live together with an enormous diversity of microorganisms, both above and below ground, which enables a very broad spectrum of interactions (Weyens *et al*., 2009). Billions of bacterial cells and thousands of different types of bacteria can occur in the root zone per gram of soil (Berendsen *et al*., 2012). For fungi, the biomass can amount to 0,5 mg per gram of soil (Bonfante & Anca, 2009). The underground fungal threads (hyphae) can be up to 100 m long and form an enormous network between plants for nutrient exchange, communication and transport (Bonfante & Anca, 2009). Remarkably, a soil without vegetation has a hundred to a thousand times fewer bacteria and fungi. If there are also contaminants, this can in turn have very strong effects on the microbial communities (quantity and diversity) depending on the concentration and nature of the contaminant (Tardif *et al*., 2016). Because bacteria and fungi in the soil play such an important role in many ecosystem processes, it is crucial to study the impact of soil contamination on microbial communities and also to evaluate the effect of remediation in terms of restoring the physicochemical soil structure but also microbiological activity.

Microorganisms enter the plant via the roots (mainly junctions between root hairs and at the level of the lateral root formation (Compant *et al*., 2010) (**Figure 3**). After they have invaded the plant, endophytes can stay in the root cortex (between the cells), or invade the xylem after translocation through the apoplast or vascular bundle system. Endophytes interact very intensively with their host plant, benefiting from a less competitive environment for nutrients and niches compared to the very diverse, complex and dynamic environment of the soil and rhizosphere.

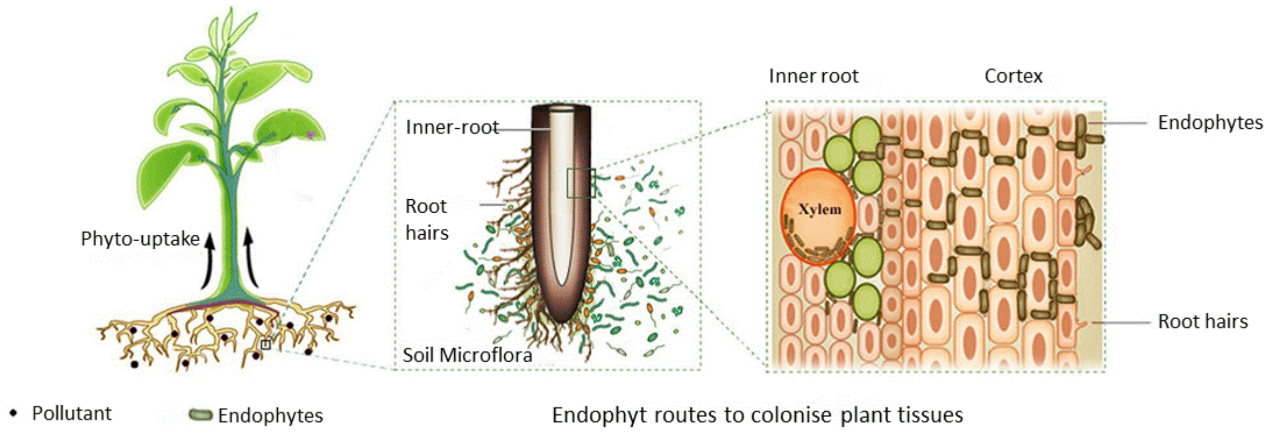


Figure 3: Colonization routes of endophytic bacteria. Adapted from Van Aken et al. (2009).

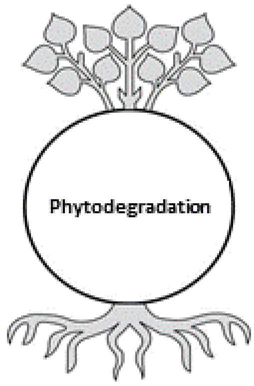
An interesting fact for the soil remediation expert is that the interactions between plants and microorganisms often lead to an improved efficiency of phytoremediation (Quiza *et al*., 2015, Thijs *et al*., 2016). Certain microorganisms can transform contaminants, fix metals and protect the plant against stress. In addition, they can also stimulate plant growth through the synthesis of plant hormones, the release of poorly soluble iron and phosphate and help with the absorption of these often limiting, non-bioavailable elements. Each plant has a specific microbiome, partly determined by the physiology of the plant and the release of a plant-specific mixture of exudates. This is an interesting but not yet fully understood phenomenon. Plant species can be chosen in terms of their interaction with certain microorganisms that can contribute more to the removal of contaminants. In addition, in the event that classical phytoremediation is not efficient enough, a selection of microorganisms can be opted for and thus transferred to microorganism assisted phytoremediation (2.2.3).

2.2.2 Standard phytoremediation mechanisms

The term "standard" phytoremediation here refers to the mechanisms that use plants and naturally occurring plant-associated microorganisms to remove or stabilize contamination. In some cases, in addition to the plants and their associated microorganisms, it is also necessary to use soil additives to reduce the availability of the contamination (for example, addition of lime during stabilization) or to increase it (only if the risks of leaching can be avoided).

The various mechanisms including the role of the plant and its naturally associated microorganisms as well as the possible need to add soil additives are described in detail below.

Phytodegradation



Phytodegradation is the uptake of contaminants in the plant and its degradation by metabolic processes in the plant (see **Figure 1**) or enzymes secreted by the plant or microorganisms (e.g. dehalogenases, nitroreductases, oxophytodienoate reductases, polyphenol oxidases, peroxidases, laccases, dehydrogenases). The contaminants are degraded into smaller molecules, in the most optimal case CO_2 which can then be released by the plant. The secreted plant enzymes often have a spatial effect, because they work on their own outside the plant, and a temporal effect: they can still be active even after the plant dies. How an enzyme works can be described as follows: polyphenol oxidase catalyses the oxidation of phenol to quinone during the breakdown of PAHs. Quinones can then condense with amino acids and peptides to form initial humic acids. A decrease in PAH concentrations with increased polyphenol oxidase activity has already been measured in Belgian soil (Andreoni *et al*., 2004).

Phytodegradation only applies to organic contaminants since metals cannot be "degraded". A first crucial aspect in phytodegradation is the **plant availability of the contamination.**

Plant associated microorganisms can play an important role to make **the contamination more bioavailable and to mobilize the contamination**. For example, there are microorganisms that produce **surfactants** that are capable of releasing oil from the soil complex. Moreover, the mobilization caused by microorganisms is not associated with the risk of leaching into the groundwater and thus further spreading of the contamination. This is in contrast to when the mobilizing agents are added as such. This difference is due to the fact that the production of mobilizing substances by microorganisms mainly takes place in the immediate vicinity of the roots and is in balance with the activity of the plant. In short: an active plant (e.g. in the summer months) stimulates microbial life in the environment of the roots, which increases the production of mobilizing agents, but at the same time the absorption capacity of an active plant is also greater. When adding mobilizing soil additives, a large part of the contamination is released at the same time, while the plant is unable to absorb all of this at the same time.

Another important step is the uptake of the contamination by the plant. As mentioned earlier, the uptake of organic molecules by the plant is largely determined by its hydrophobicity (log K_{ow}). Various chlorinated solvents, herbicides, pesticides, insecticides and explosives are well taken up by plants. However, efficient availability and uptake of the contamination is not yet a guarantee for a successful phytodegradation. Once the contamination has been taken up, only a sufficiently high degradation efficiency in the plant can ensure that both phytotoxicity and any volatilization can be avoided. Here too, the microorganisms play a crucial role. It is difficult to say which microorganisms (species, functional groups) are important for the degradation of a certain contaminant. Often the degradation elements are on plasmids (small circular pieces of DNA in a bacterium) that can be exchanged freely.

A few examples: a recent study has shown that a community dominated with *Pseudomonas sp.* was efficient to degrade diesel, but if this group was selectively killed and *Burkholderia sp.* could dominate, a faster degradation was found (Bell *et al*., 2013). This indicates that there is functional redundancy in microorganisms to efficiently remove a contaminant. Remediation experts can use inoculants with degrading microorganisms (genes) to make up for deficiencies at a certain site (see 2.2.3). The KEGG database can be consulted to search for specific microbial genes for the degradation of aromatics (http://www.genome.jp/kegg/pathway.html).

Rhizodegradation

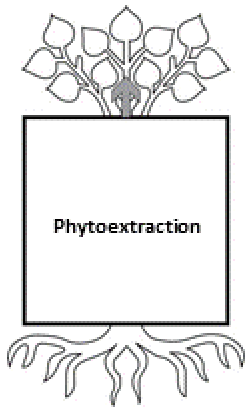
Rhizodegradation is a process similar to phytodegradation, the contaminants are degraded but here mainly through microbial activity in the soil in the immediate vicinity of the plant roots. The term rhizodegradation is mainly used when it concerns degradable contaminants that cannot be taken up by the plant. However, with every application of phytodegradation (degradation in the plant), part of the contamination is also degraded by rhizodegradation (degradation in rhizosphere, outside the plant). Although absorption by the plant is not a requirement for rhizodegradation, good availability is still necessary. Similar to phytodegradation, microorganisms play an important role here.

In the rhizosphere, microbial activity is stimulated by the presence of plant roots and by the supply of oxygen, water and rhizodeposits.

These rhizodeposits are residual products of photosynthesis (5 to 20% of the net carbon fixed by a plant is released into the rhizosphere). It includes both inorganic components (CO_2 from cell respiration and proton efflux) and a wide range of complex organic components (such as cellular remains, tissues, mucus and proteins) and the (in)soluble low molecular weight components (also known as exudates) such as different classes of sugars, amino acids, amides, organic acid aromatics and phenols.

In addition to a rich food source for microorganisms, some of these secreted compounds may act as inducers for the breakdown of aromatic components by bacteria. An additional advantage of rhizodegradation is that the microorganisms present in the rhizosphere can spread through the soil faster if they are adsorbed to the roots. In this way microorganisms adapted to the contamination can colonize a larger volume of contaminated soil more quickly. This process is important for, among other things, the degradation of petroleum hydrocarbons (Van Hamme *et al*., 2003) and explosives (Rylott *et al*., 2011).

Phytoextraction



In phytoextraction, the contaminants, often metals, are taken up by the plant tissues and subsequently accumulated in preferably the above ground parts of the plant (**Figure 1**). The metal uptake and accumulation are highly dependent on the plant species, the type and concentration of the contamination, the pH and the bioavailability of the metals in the soil. There are various mechanisms that play a role in the uptake of metals by plants, f.e. exudation of protons and organic acids promotes the bioavailability and mobilization of metals in the rhizosphere.

In addition, plant associated chelators such as phytochelatins and siderophores can form metal complexes, after which they can be taken up by the plant and subsequently transported and translocated (**Figure 4**). Metal-tolerant microorganisms can promote the extraction of metals through the secretion of acids and H^+, they can cause the detoxification of the metals, and improve the plants biomass production as well as reducing stress. The main advantage of this microorganism induced mobilization of the metals is the fact that this happens in balance with the activity of the plant. As a result, greater mobilization is always accompanied by higher uptake, so that risks of leaching are avoided.

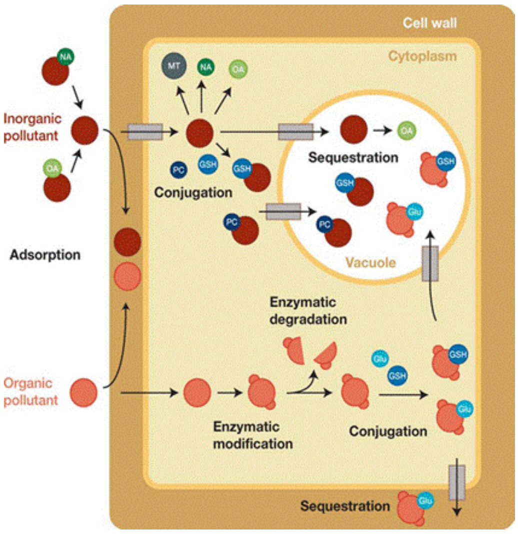


Figure 4: Mechanisms for the uptake and storage of organic and inorganic contaminants, adapted from Pilon-Smits, 2005. PC: phytochelatins, OA: organic acids, GSH: glutathione, MT: metallothioneins, NA: nicotianamine, Glu: glutamic acid.

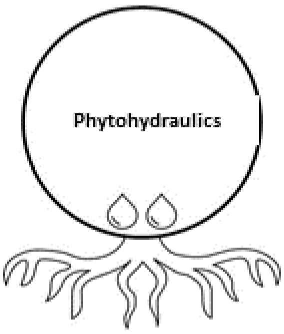
The mobilisation of metals by using soil additives, is accompanied by high risks of leaching into the groundwater because a too high concentration of metals is released at the same time and the plant cannot absorb it quickly enough. Mobilizing soil additives can therefore only be used to accelerate phytoextraction if the risks of leaching can be avoided.

Many examples are known of endophytic microorganisms that have extremely high tolerances for metals such as Cr, Cd, Cu, Pb, Zn and that stimulate both plant growth and the uptake of metals by the plant. However, not many studies are known that describe the total microbiome of metal-accumulating plants (Thijs *et al*., 2017). More than 99% of the bacterial communities of hyperaccumulators have yet to be described for the first time.

Plants that accumulate the metals also have to be harvested often. This must be done carefully so that the metal containing biomass does not contaminate the soil again when the plants die or trees lose their leaves. There are various economically viable ways to value the metal containing biomass, for example by pyrolysis.

Phytoextraction is typically applied to inorganic contaminants, such as metals, metalloids, and radioactive elements. Phytoextraction usually takes a long time (ten years depending on the degree of contamination, the remediation objective that must be achieved and the condition of the site to be remediated). That is why phytoextraction is often seen as a secondary benefit, alongside other actions for nature restoration and soil valorisation.

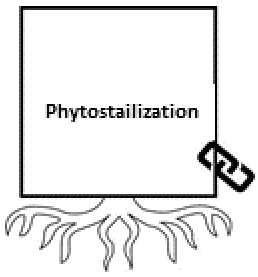
Phytohydraulics



Phytohydraulics is based on the capacity of plants to transpire (evapotranspiration) surface and groundwater (**Figure 5a and 5c**). The horizontal migration of groundwater can be controlled and contained by deep-rooting plant species that can absorb and transpire a lot of water. Trees that are classified as phreatophytes are deep-rooted, rapidly transpiring trees that prefer wet soils and can tolerate temporary periods of water saturation. Typical phreatophytes are poplars and willows. Trees rooted in a contaminated groundwater plume can limit the spreading of the contamination, a so called barrier or groundwater plume containment technique. Poplars can also be planted at sites with contaminated groundwater and function as a "groundwater treatment system".

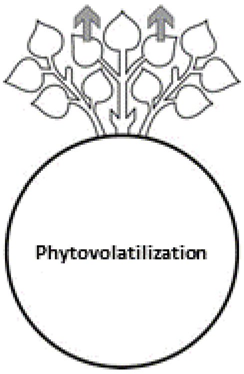
Phytostabilization

Phytostabilization is the process in which plants are used to immobilize contaminants by adsorption, absorption and accumulation in the roots, precipitation in the root zone or by physical stabilization of the soil (**Figure 5d**). Phytostabilization reduces the spreading of contaminants into the groundwater or the atmosphere. It can, among other things, play a useful role in capturing metals and hydrophobic hydrocarbons in the soil (**Figure 4**).



Over time, the concentrations of bound hydrophobic hydrocarbons may decrease due to microorganism assisted degradation. A common problem with phytostabilization is excessive phytotoxicity. In this case it is advisable to work with stabilizing soil additives (for example, lime, phosphates, mineral oxides and organic substances) in order to limit the fraction of contamination that the plant can absorb, and therefore also phytotoxicity.

Phytovolatilization



After uptake by the plant, the contaminants are transported to the leaves of the plant (**Figures 5a and 5c**). Volatile contaminants are excreted by evaporation.

The plants create accelerated evaporation so that the removal of contaminants increase. However, it can cause a shift of the problem to another environmental compartment and this must therefore be properly monitored and avoided. Phytovolatilization can therefore only be applied if the volatile contamination is rapidly degraded once it enters the air or if the release can occur under controlled conditions. However, in the case of degradable volatile contamination, it is advisable to ensure that sufficient degradation capacity is present so that phytodegradation and rhizodegradation occur instead of phytovolatilization. Endophytic microorganisms play an essential role in the degradation of water-soluble volatile components. To prevent the potential problem of phytovolatilization, it is therefore recommended to add the correct microorganisms to the plant via inoculation (see 2.2.2).

Table 1: Presentation of phytoremediation mechanisms. Table adapted from Interstate Technical Regulatory Council (ITRC). 2009. Phytotechnology Technological and Regulatory Guidance and Decision tree.

Mechanisms	**Description**	**Remediation goal**
Phytodegradation	Process in which plants and their associated microorganisms absorb and degrade contaminants in plant tissues through metabolic processes or enzymatic activity.	Remediation by degradation
Phytoextraction	Process in which plants and their associated microorganisms absorb contaminants and fix them in plant tissue.	Remediation by removal of plants that have taken up the contamination
Phytohydraulics	Process in which plants and their microorganisms absorb and transpire water and thereby influence the groundwater level and the direction and speed of the groundwater flow.	Containment by controlling hydrology
Phytostabilization	Process in which plants and their microorganisms fix certain contaminants in the rhizosphere or in the roots.	Reduction of spreading of contaminants into the groundwater or the atmosphere
Phytovolatilization	Process in which plants take up the contaminants and transport them to the leaves of the plant. The volatile contaminants are subsequently excreted by evaporation	Remediation by transpiration of plant
Rhizodegradation	Process in which contaminants are degraded in the rhizophere by a combination of plant enzymes and microorganisms in the rhizophere.	Remediation by degradation

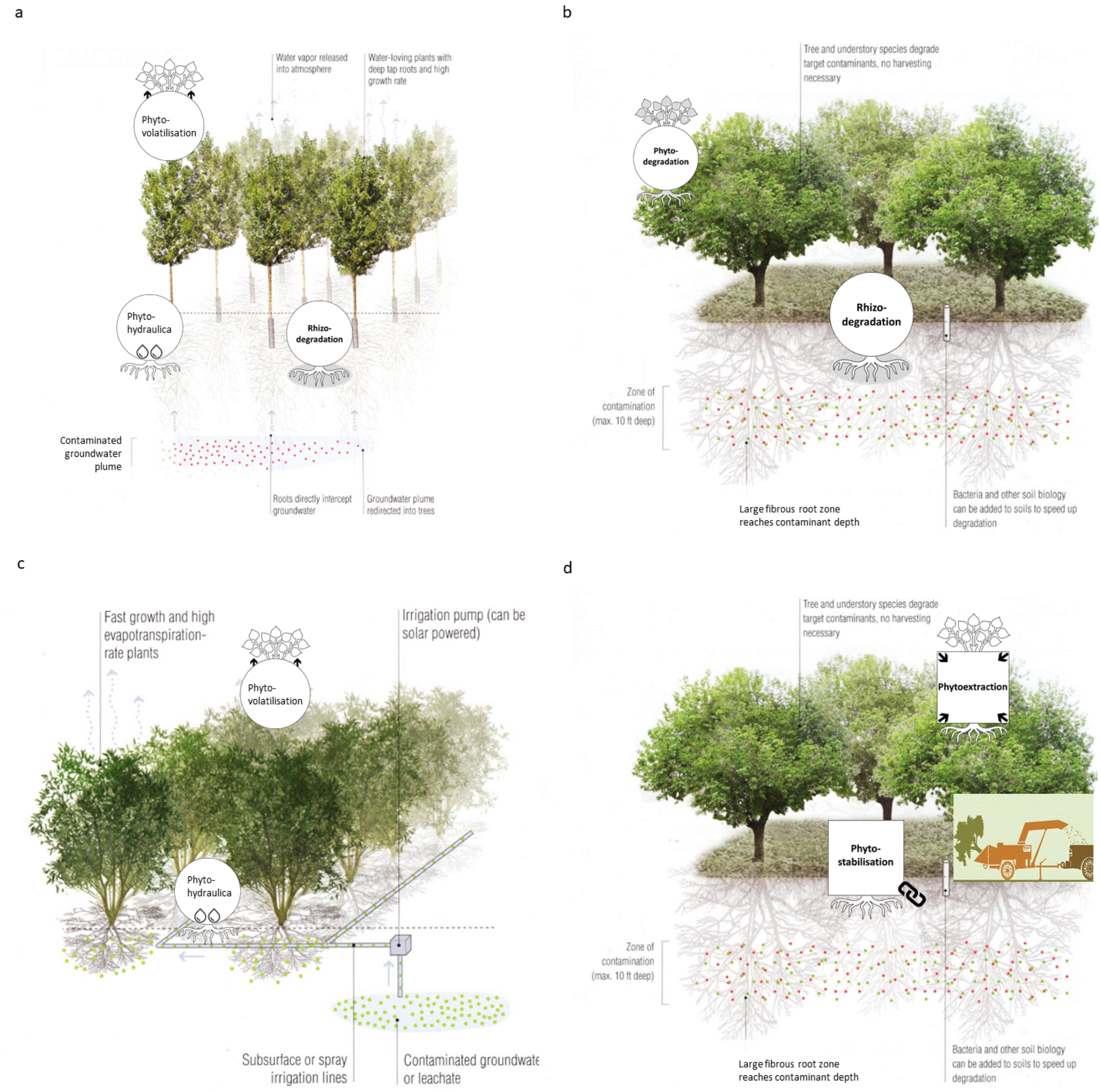


Figure 5: Simplified sketches of phytoremediation mechanisms. Adapted from "PHYTO, Principles and resources for site remediation and landscape design", by Kate Kennen and Niall Kirkwood, 2015.

2.2.3 Microorganism-assisted phytoremediation

In case the remediation objectives cannot be sufficiently achieved with standard phytoremediation, microorganism assisted phytoremediation can offer a solution. In microorganism-assisted phytoremediation, specially selected microorganisms are enriched in the rhizosphere and/or within the plant to accelerate the degradation of organic contaminants, to improve the extraction or stabilization of metals and to promote plant growth and health (Weyens *et al*., 2009, Weyens *et al*., 2009, Thijs *et al*., 2016). Another term that is used is bioaugmentation. Adding microorganisms is not a remediation in itself but must be seen as a supportive measure. The growth of these microorganisms can also be optimised by the addition of electron acceptor, nutrients. The bacteria used for bioaugmentation are preferably **isolated from the site itself**, because more good interactions occur between naturally occurring microorganisms and local plants than with site-foreign microorganisms (Bell *et al*., 2014b). The same applies when working with foreign plants, a symbiosis with the host plant must be possible, and these interactions are stronger between native plants and naturally occurring microorganisms (Bell *et al*., 2014b).

The **difference between bacteria assisted phytoremediation and bacteria assisted bioremediation** is that in phytoremediation, the plant creates a **favourable** environment whereby introduced bacteria will remain present much longer and can exert their effect longer, in contrast to location without plants where introduced bacteria become very quickly overgrown by the microorganisms present (Bento *et al*., 2005). The **successful application of bioaugmentation** is only possible if account is taken of the time of inoculation, the inoculation method and above all one must have a good understanding of the ecological interactions that determine whether a symbiosis between plants and degrading microorganisms is 'viable' (Thijs *et al*, 2016). That is why it is recommended to work together with phytoremediation specialists for bioaugmentation.

Bioaugmentation can be used for all organic and metal contaminants. There are **various ways in which microorganisms can contribute to optimize phytoremediation**.

1) Microorganisms can promote general plant growth and development, which can be crucial, for example, to suppress the negative effects of phytotoxicity.
2) Microorganisms can stimulate the growth and development of a more extensive root system, which in turn results in an increased radius of influence of the plant for the contamination.
3) The promoting of plant growth can also increase pumping capacity, which is advantageous in phytohydraulics and phytovolatilization applications.
4) Microorganisms can interact with the contaminants in different ways: they can provide mobilization (e.g. through the production of surfactants and organic acids), stabilization (e.g. through the production of chelators), degradation and detoxification (e.g. through sequestration on the cell wall).

Table 2 shows for each phytoremediation mechanism (i) when it is appropriate to **switch from standard phytoremediation to microorganism assisted phytoremediation** and (ii) which processes can be promoted by enriching certain selected microorganisms.

Table 2: Standard phytoremediation vs. microorganism assisted phytoremediation and the role of the microorganisms added

Phytoremediation mechanism	**When is it appropriate to switch from classical phytoremediation to microorganism assisted phytoremediation?**	**Microorganisms that are enriched are responsible for promoting:**				
		General plant growth	Stabilization	Mobilization	Degradation	Detoxification
Phytodegradation	Insufficient degradation potential Volatilization via the leaves Insufficient availability Insufficient uptake Phytotoxicity	x		x	x	x
Rhizodegradation	Insufficient degradation potential Insufficient availability Phytotoxicity	x		x	x	x
Phytoextraction	Insufficient availability Insufficient uptake Insufficient translocation Phytotoxicity	x		x		x
Phytohydraulics	Insufficient biomass Insufficient pump capacity Phytotoxicity	x	x			x
Phytostabilization	Too much availability Too much uptake Phytotoxicity	x	x			x
Phytovolatilization	Insufficient availability Insufficient uptake Phytotoxicity	x		x		x

In microorganism assisted phytoremediation, the most beneficial microorganisms for the active phytoremediation mechanism are selected and enriched via inoculation (**Figure 6a**). In many cases, the inoculation will also have to be repeated several times to ensure the presence of the inoculated microorganisms. In some cases, consortia (groups of microorganisms) can also be used that can better maintain and establish themselves in the soil under controlled conditions.

Figure 6: a) Inoculation of poplar at a kerosene-contaminated site. b) Sampling and measurement of trichloroethylene evapotranspiration through poplars. Photo: Nele Weyens, Hasselt University.

An important strategy that can be applied to increase the success of colonization is to use **endophytes**, bacteria that live in the plant in the intracellular spaces or in the plants xylem and phloem without negative effects for the plant. The "environment" in the plant is less stressful for microbes, there is a lower biodiversity and therefore less competition between microorganisms, which can increase the success of establishing specific bacteria. A lot of studies are currently being carried out on the presence of endophytes in a certain plant species. This can also facilitate the selection of endophyte species for inoculation (Beckers *et al*., 2016). Currently, there are strong indications that endophyte communities are to a large extent specific to a plant species (and even cultivar), and to some extent determined by environmental factors including soil microorganisms (Berg & Smalla, 2009). Endophytes equipped with trichlorethylene and toluene degradation genes inoculated into the root zone of poplars colonized the tree quickly, and it was even found that the degradation genes were transferred via **natural gene exchange** to other natural endophytes that did not yet have these genes (Barac *et al*., 2004, Taghavi *et al*., 2005, Weyens *et al*., 2010).

The result was that much more toluene or TCE was degraded to CO_2 and H_2O and therefore no harmful residual products were evapotranspirated. This technology therefore has a very high potential and can be used for many other organic substances. The root endophyte microbiome of Acer pseudoplatanus, an important tree species for the remediation of contamination with explosives, has also recently been studied in detail (Thijs *et al*., 2018).

2.2.4 Positioning of (microorganism-assisted) phytoremediation relative to other remediation techniques

Since phytoremediation is based on natural processes, a comparison is often made with **natural attenuation** (reduction of contamination by natural processes). More specifically: What is the added value of phytoremediation compared to natural attenuation?

The main difference lies in the fact that when applying phytoremediation you select a specific plant. In case of a groundwater contamination, it will be necessary to plant phreatophytes since the roots of these trees go into the groundwater. When a superficial contamination is present, grasses with more superficial roots will be selected. For many contaminants it is known which plants (species/cultivars) are able to accumulate which contaminants. A **good plant choice** can therefore significantly improve efficiency. Finally, when planting for phytoremediation, a **drainage system** can be provided that makes it possible to further promote the phytoremediation process by adding specific microorganisms. If no vegetation cover is present in case of natural attenuation, the presence of the plant in phytoremediation offers additional advantages, the most important being **the oxygen supply in deeper soil layers** via the plant roots, **the stimulation of the microbial activity in the root zone** and the **'attraction' of the contamination**.

Bioremediation is also often compared to phytoremediation. However, the use of plants has many, often insufficiently known, advantages. For example, the root system **strongly promotes the oxygen supply** to the soil, which in most cases has a positive effect on the degradation of organic contamination. Furthermore, the **root exudates provide a strong stimulation of microbial activity** in the area of the roots. Depending on which microorganisms are involved, this may result in (i) higher availability of the contamination or (ii) improved degradation of the contamination. Because the **higher availability occurs in the immediate vicinity of the roots** of the plant, the contamination made available can be taken up directly by the plant, thus preventing the risks of leaching into the groundwater. Finally, plants can have an enormous **pumping capacity**. A willow tree can transpire up to 200 l of water per day. This enormous pumping power creates an additional attraction of contaminants.

Finally, phytoremediation, more specifically phytodegradation and rhizodegradation, can be compared with a conventional “**pump and treat**” remediation. The plants present act as a **solar-powered pump**, while the plant associated microorganisms cause the degradation of the contamination. It should be noted here that the highly dispersed root system of plants ensures that the radius of influence of contamination by the plant is much larger than with a conventional pump. Moreover, the roots of phreatophytes can go up to 10 m deep to reach the groundwater. The degradation is, as already indicated above, promoted by an additional oxygen supply and stimulation of microbial activity in the vicinity of the roots.

2.2.5 Phytotechnology applications in practice

Phytotechnology applications can be classified based on the fate of the contaminant (degradation, extraction, immobilization, volatilization or a combination) (**Table 1**, **Table 2** and **Figure 5**). Phytotechnology applications can also be classified based on the mechanism involved such as extracting contaminants from the groundwater by phreatophytes, concentrating the contaminants in the plant tissue, volatilizing volatile substances from the plant to the air , the immobilisation of the contamination in the root zone, the control of leaching through buffer zones and the control of infiltration through a vegetation cover.

Table 3: Phytotechnology applications

Application	Medium	Mechanism
Vegetation covers for stabilization of soil, sediment and for infiltration control	Soil Sediment Groundwater Surface water	Phytodegradation Phytoextraction Phytohydraulics Phytovolatilization Rhizodegradation Phytostabilization
Vegetation covers for remediation	Soil Sediment Groundwater Surface water	Phytodegradation Phytoextraction Phytohydraulics Phytovolatilization Rhizodegradation Phytostabilization
Hydraulic barriers	Groundwater	Phytoextraction Phytodegradation Phytohydraulics Phytostabilization
Trees for phytoremediation	Soil Sediment Groundwater	Phytodegradation Phytoextraction Phytohydraulics Phytovolatilization Rhizodegradation Phytostabilization

Application	Medium	Mechanism
River bank buffers and buffer zones along motorways and fields	Soil Sediment Surface water Groundwater	Phytodegradation Phytoextraction Phytovolatilization Rhizodegradation Phytostabilization
Biofilters (reedlands, swamps and other ‘constructed wetlands’)	Sediment Surface water	Phytodegradation Phytoextraction Phytovolatilization Rhizodegradation Phytostabilization

Vegetation covers for soil/sediment stabilization

Soil and sediment can spread (vertically and laterally) when exposed to uncontrolled water flows and/or mobilization by wind, in particular “erosion” or “leaching”. Stabilizing vegetation offer a natural barrier and resistance to erosion and leaching.

The most important mechanism that helps to prevent erosion is the infusion of plant roots into the soil or sediment. Plants with fibrous root systems are typically used, such as grasses, herbaceous species and water-rich species. When the soil or sediment is contaminated, the contaminants can also be addressed by plants. Specifically, phytostabilization refers to soil/sediment erosion or to minimize bulk migration of the contamination, while phytosequestration mechanisms address the mobility of the contamination itself. In vegetation covers for soil or sediment stabilization, plants have been specifically selected to control soil/sediment migration (via infusion with fibrous root systems) and/or to prevent migration of contaminants by phytosequestration. In some cases, the same plant species can serve both purposes.

In addition, other plants can be selected based on their phytoextraction capacity and hence accumulate contaminants in the aboveground plant parts.

Vegetation covers for infiltration control

Vegetation covers for infiltration control use the ability of plants to absorb significant volumes of water and to minimize the infiltration of the contamination (Veissman, Lewis and Knapp 1989).

The most important phytotechnology mechanism for these applications is phytohydraulics (see Section 2.2.2). Vegetation covers for infiltration control use plants that maximize the evaporation and plant evapotranspiration processes of the system. The vegetation usually consists of seed mixtures or combinations of plants/trees that have access to the water and create a hydraulic barrier.

Vegetation covers for remediation

In addition to the ability of cover systems to stabilize soil/sediment and the application of a hydraulic control, densely planted soil coverings and grasses can also be used to remediate contaminants. Remedial covers are vegetation systems that are generally applied to soils with very superficial contamination (contamination in the top layer of the soil). On the contrary, phytoremediation with trees (see section 2.2.2) are applied for soils and/or groundwater with deeper contaminants.

The typical radius of influence of effectiveness for remedial covers is 30 to 60 cm below the ground surface; however, in some situations it was reported that depths up to 1.5 m were influenced by these vegetation covers (Olsen and Fletcher 1999).

Phytoremediation includes rhizodegradation, phytodegradation and/or phytovolatilization mechanisms (see sections 1.2.2, 1.2.5 and 1.2.6 respectively) to reduce the concentrations of contaminants at the site.
In addition, phytoremediation also includes phytoextraction (see section 1.2.4) as long as the harvesting and removal of the contaminants is included in the application. Soil covers for phytoremediation are (in some countries) widely applied to soils contaminated with PAHs, PCBs and other persistent organic contaminants that are generally less mobile, less soluble, less biodegradable and less available. Reviews of these works can be found in literature (Flathman and Lanza 1998; Frick, Farrell and Germida 1999; Zeeb *et al*. 2006; Russell 2005).
Finally, remedial soil covers are used for phytoextraction of specific inorganic contaminants such as metals, salts and radionuclides. Typical concentration ratios of many such elements have been described by many scientists (Wang, Biwer and Yu 1993). The aboveground parts of the plant where the inorganic contamination accumulates must be harvested by conventional farming methods and must be removed from the site.

Hydraulic barriers

Hydraulic control is another form of control of a groundwater contamination plume or for the removal of groundwater that flows into uncontaminated area (Ferro *et al*., 2003, 2013). Trees can remove a substantial amount of groundwater from the contaminated zone by using the groundwater through transpiration and thereby act as biological pumps. The depth of the groundwater table relative to the root depth is an important consideration. Some tree species that root deeply are extremely suitable for hydraulic control. Hydraulic control can be calculated and modelled by comparing the amount of transported water with the groundwater flow, and by taking into account site specific, geological and climatic variables.

Planting of trees for phytoremediation

In addition to the ability of deep rooted plants and trees to absorb and transpire groundwater, they can also be used for phytoremediation of deeper soil layers and contaminated plumes that are located, for example, at the top of the groundwater level. Phytohydraulics can be used to bring the contaminants into the root zone through the pumping effect of the trees. Rhizodegradation, phytodegradation and/or phytovolatilization mechanisms (see section 2.2.2) can remediate contaminants in the unsaturated zone.

Phytoremediation with trees are applied (internationally) on a large scale to groundwater contaminants with substances such as petroleum products (BTEX, MTBE, aliphates, mineral oil) and chlorinated hydrocarbons. The lighter fractions of these components are generally mobile, soluble and biologically available with log K_{ow} values in the range where uptake into plants is expected. But the bioactivity in the rhizosphere also plays a major role since the contaminants can often be degraded here before they are taken up by the plant. Various reviews of these applications have been published (Chappell 1998, Van Den Bos 2002). In addition, some plantations of trees for phytoremediation have been successful even when planting in a free-phase product zone. A drastic reduction of the concentrations was observed as the contamination plume flowed through the root zone (Fiorenza *et al*. 2005, Nichols *et al*., 2014). Willows and poplars that were planted on a petroleum contaminated groundwater plume were able to successfully reduce the contamination plume, extracting groundwater from 23 litres to 59 litres per day per tree (Ferro *et al*., 2003, 2013).

Riverbank buffers and buffer zones along motorways and fields

Riverbank buffers are areas covered with vegetation that protect adjacent water sources against contamination. In addition, these buffers offer protection against erosion and are a habitat for aquatic animals and other wildlife.

Riverbank buffers are known to be of vital importance for hydrology control, for the cleaning of drainage and shallow groundwater. The hydrology is influenced by the vegetation in the bank buffer with the same mechanisms that control phytohydraulics, while their root systems promote phytosequestration, rhizodegradation, phytoextraction, phytodegradation and/or phytovolatilization (see section 2.2.2).

Biofilters (reedlands, marshes and other “constructed wetlands”)

Reedlands, marshes and other biological treatment systems use geochemical and biological processes that are inherent in the 'wetland' ecosystem to remove metals, explosives and other organic contaminants from waste water. The main processes that take place are filtration and rhizodegradation. Although phytotechnology includes all components of the ecosystem: organic soils, vascular plants, algae, and microbial fauna, it is primarily the microbial activity that is responsible for the remediation.

Waste water with high concentrations of heavy metals flows through the aerobic and anaerobic zones of the biofilter. The metals are removed by ion exchange, adsorption, absorption and precipitation with geochemical and microbial oxidation and reduction (e.g. precipitation of metals such as hydroxides and sulphides). Ion exchange occurs when the metals make contact with humus or other organic components in the reedland. The metals precipitate and remain bound in the swamps or are filtered out as the water percolates through the biofilter or is taken up by the plants. In the latter case, the plants that have taken up metals can be used as a raw material for pyrolysis where biogas and bio-oil can be recovered and the small residual amount with metal containing biochar can be safely removed.

An engineered reed where gravel was replaced by material such as coal, activated carbon, graphite resulted in water treatment of organic substances up to five times faster than a conventional reedland(Aguirre-Sierra *et al*., 2016). This is because the specific material can accelerate electron transport in the anaerobic zone and thus also the microbial metabolism of electrogenic bacteria (e.g. *Geobacter sulfoaducens*) that remove the ammonium and reduce the biological oxygen demand (BOD). If the waste water treatment basin is also electrically polarized, the degradation of organic contaminants occurs even faster (iMETLand, 2020; http://imetland.eu/).

2.3 CONTAMINANTS

Phytoremediation can be applied to a wide variety of contaminants, each with their own specific characteristics and boundary conditions. Often the type of contamination determines which mechanism can be applied, but also which possible bottlenecks must be taken into account. The efficiency of phytotechnology also strongly depends on which plants are selected for remediation and which control measures are taken. Results from laboratory studies, greenhouse experiments, pilot tests and real field experiments on similar sites can be a very important guide in determining whether a phytotechnology is suitable for a particular site. See further under chapter 3. Success stories can help you make the right plant choice for application at new contaminated sites. See also section 2.4 and chapter 3. If relevant local data are not available then a pilot test or area-specific test is certainly required.

Due to the diversity of contaminants that theoretically are applicable for a phytoremediation technology, they are subdivided into large categories of organic and inorganic contaminants. **Table 5** shows which phytotechnology mechanism is involved for each category of contaminants, which applications have already been implemented and proved successful, the scale on which it has already been applied and a brief explanation of the most important findings and references. In addition, based on this extensive literature study and an expert evaluation for all contaminants, an estimate was made of the phytoremediation potential. For this, the duration and the possibility of upscaling to field applications were used as the most important criteria (**Figure 8**).

2.3.1 Organic contaminants

Many organic contaminants can be degraded or stabilized by phytodegradation, rhizodegradation and phytostabilization. In addition, phytohydraulics can also be used to limit spreading of groundwater (contamination) and/or remediate contaminated groundwater. Information about how phytotechnologies can be applied for certain organic substances is detailed in the following sections.

Chlorinated solvents and volatile organic components

Deep rooted poplars have repeatedly proved to be very successful in tackling groundwater contaminations with chlorinated solvents or BTEX, by means of rhizodegradation, phytodegradation, and sorption of the substances on the plant tissue (**Figure 4**) (Porteous Moore *et al*., 2006). The release of the volatile fraction of the contaminants into the atmosphere must be avoided through enrichment of degrading bacteria in the root zone and inside the plant (Barac *et al*., 2004, Newman & Reynolds, 2005, Taghavi *et al*., 2005, van der Lelie *et al*. al., 2005). The inoculated bacteria can neutralize the volatile toxic substances because they possess the specific degradation genes. Moreover, these bacteria can pass on the degradation genes to bacteria that naturally live in the stem and leaves of the plant (endophytes) and that did not yet have these genes. The endophytes equipped with the degradation genes can now also degrade the volatile toxic substances as they "flow" through the vascular bundles. Due to the longer contact time (hours, days), therefore, complete mineralization to CO_2 and H_2O can take place that is released through the leaves. The same strategy was successfully applied in situ to remediate trichloroethylene (TCE) contaminated groundwater (Weyens *et al*., 2009, Weyens *et al*., 2010, Weyens *et al*., 2010, Weyens *et al*., 2015). Only for BTEX it was sufficiently demonstrated that the naturally occurring degradation capacity is sufficient to prevent volatilization, so that it can be concluded that classical phytoremediation is feasible for this contamination. For the other chlorinated solvents and volatile organic components, however, it is necessary to demonstrate that there is sufficient degradation potential to prevent volatilization and to minimize phytotoxicity.

Munition and explosives

Phytotechnologies are also promising for the remediation of areas contaminated with explosives, mainly for the remediation of large areas, slightly contaminated and diffusely dispersed soil and groundwater contamination (Ramos *et al*., 2005, Rylott & Bruce, 2009). The Ministry of Defence has already shown an interest in the use of rhizodegradation for the remediation of soils contaminated with explosives including trinitrotoluene (TNT) propellant gases such as dinitrotoluene (DNT) and similar components.

Studies have already been carried out into grasses and maple trees for stabilization and rhizodegradation of contaminants with explosives. For example, the microbial community in the root zone of grasses on the Helchteren firing range was studied. It was established that the bacteria in the root zone could rapidly degrade the propellant gas DNT (Thijs *et al*., 2014). The presence of the bacteria was also important for the growth of grasses and the recovery of the non-overgrown or "dead zones" (**Figure 7**).

Figure 7: Bare "dead zone" at the Helchteren firing range. Photo: Sofie Thijs, Hasselt University.

Studies were also conducted into the optimization of the rhizodegradation of the highly degradable and resistant explosive TNT in the soils of the fort at Zwijndrecht. The maples on the site contain bacteria in their roots with enzymes that could denitrate TNT, i.e. split off a nitrogen group, which could subsequently be used by the bacteria as an N source and thus reduce the toxicity of TNT (Thijs *et al*., 2014a, Thijs *et al*., 2014b).

Persistent organic substances (POP)

POPs consist of a group of substances mainly pesticides and polychlorinated biphenyls with the following characteristics: they are toxic, persistent, bioaccumulating and can be transported over very large distances. Phytotechnologies are generally difficult for PCB-contaminated soils, but can be used as aftercare to remove pesticide residues (Eevers *et al*., 2017).

Field studies showed that zucchini and pumpkins (*Cucurbita sp.*) could absorb pesticide residues in the roots and also transport them to the shoots (White *et al*., 2003, Wang *et al*., 2004). A pilot study in the US compared the possibility of 21 zucchini varieties of 2 types of *C. pepo ssp texana* and *C. pepo ssp pepo* for the inclusion of dichlorodiphenyldichloroethane (DDE) and the effect of inoculation with suitable bacteria on the growth and health of the plants. Results showed that the *C. pepo ssp pepo* variety extracted three times more DDE from the soil (White *et al*., 2003), and this was further improved after inoculation with a consortium of plant growth stimulating and DDE-degrading endophytes (Eevers *et al*., 2017). The inoculated plants also did not show white disease, while this was the case with the non-inoculated plants. Soil samples taken after one growing season already showed a significant reduction in DDE concentrations in the soil. Experiments were also conducted with copper nanoparticles. The results of these lab studies showed that copper nanoparticles greatly increased the uptake of DDE by the plant, but the underlying mechanisms still need to be studied (De La Torre-Roche *et al*., 2013).

POPs were also addressed through phytostabilization, phytohydraulics, phytodegradation and phytoextraction (Zhu *et al*., 2014, Arslan *et al*., 2015). Studies have also been conducted on transgenic plants that can phytoremediate sites contaminated with POPs (Sylvestre *et al*., 2009). The plants were equipped with an enzyme isolated from bacteria to degrade PCBs and reduce the toxicity of chlorinated compounds.

Mineral oil and PAHs

Mineral oil is a collective name for a number of products that consist of various distillation fractions of petroleum: gasoline, diesel, kerosene, motor oil and fuel oil. The chemical composition of 'mineral oil' can vary considerably and therefore also have effects on the possibilities for phytoremediation. A thorough knowledge of the composition of the mineral oil is therefore essential to be able to estimate the feasibility of phytoremediation and the possible remediation objectives. The determination of the composition of mineral oil on the basis of the EPK/VPK method or oil characterization is often already carried out as part of the risk assessment in the descriptive soil survey (Human risk assessment for mineral, OVAM 2007).

Table 4: Overview of the most important indicator substances for the most common mineral oil contaminations in soil and groundwater. (From Human risk assessment for mineral, OVAM 2007)

Indicator substances	**Benzene**	**Kerosene**	**Diesel, light heating oil**	**Heavy heating oil**	**Crude oil**	**Lubricating oil**
BTEX	X	X				
PAH		X	X	X	X	X
Aliphates						
C5-C6	X				X	
C6-C8	X	X	X	X	X	
C8-C10	X	X	X	X	X	
C10-C12	X	X	X	X	X	
C12-C16		X	X	X	X	
C16-C21			X	X	X	X
Aromatics						
C8-C10	X	X	X	X	X	
C10-C12	X	X	X	X	X	
C12-C16	X	X	X	X	X	
C16-C21		X	X	X	X	
C21-C.35				X	X	X

Extensive studies (laboratory and greenhouse experiments) on soil samples from sites contaminated with mineral oil have shown the degradation (due to rhizodegradation) of mineral oil in these soils (Ramos *et al*., 2010, Balseiro-Romero *et al*., 2017, Yateem, 2013). In general, studies on rhizodegradation of mineral oil make use of grasses, poplars, willows, but also leguminous plants (Gkorezis *et al*., 2016, Kaimi *et al*., 2007). The presence of mixtures of contaminants on sites contaminated with mineral oil does not always make it easy to develop a phytoremediation project and choose the correct application. High-molecular weight polycyclic aromatic hydrocarbons (PAHs) are less bio-available and more difficult to remediate through phytotechnology alone. To increase bioavailability, surfactants can be added, but soil bacteria can also produce **biosurfactants** that reduce surface tension and make it easier to absorb and degrade the contaminants (Borah & Yadav, 2017). The composition of the mineral oil is also an important factor. The lighter C6-8, C8-10, C10-12, and C12-16 fractions are more readily biodegradable by microorganisms than the heavier fractions (Balseiro-Romero *et al*., 2017). Biodegradation of n-alkanes with chain lengths up to C44 has already been demonstrated (see Code of good practice, natural attenuation). N-alkanes can be degraded via various oxidation mechanisms. In oxidation, the initial oxidation step is carried out at one of the ends of the carbon chain by a mono-oxygenase, first forming a free radical and then an alcohol, which is further oxidized to an aldehyde or carboxylic acid. By β-oxidation of the carboxylic acid, fatty acids and acetyl coenzyme A are formed, with the ultimate release of carbon dioxide.

Laboratory and greenhouse experiments have shown that alkanes and the C10-14 fraction can be degraded by combinations of different grasses and rhizodegradation, while deep rooting poplars and willows tackle the deeper contamination with phytodegradation and rhizodegradation (Ramos *et al*., 2010 Balseiro-Romero *et al*., 2017, Yousaf *et al*., 2010, Yousaf *et al*., 2011, Page *et al*., 2015). Rhizodegradation leads to faster and more complete remediation than bioremediation (Gkorezis *et al*., 2016, Khan *et al*., 2013, Mezzari *et al*., 2011) due to the greater density and activity of microorganisms in the vicinity of plants. In the case of petrol and kerosene, based on extensive experience, it can be concluded that phytoremediation is feasible as an active remediation technique. For diesel and light fuel oil, phytoremediation can be applied if sufficient degradation potential is guaranteed, while for the heavier fuel oil and other contaminants and mixtures a thorough feasibility analysis is required before conclusions can be drawn (see also chapter 3 for more explanation).

2.3.2 Metals and other inorganic contaminants

Metals and other inorganic contaminants in the soil cannot be degraded, but can be stabilized or extracted. There are many plants that can absorb, transport and store heavy metals in the aboveground biomass, but this process is generally slow. It is therefore recommended to combine phytoextraction with economic valorisation of the biomass for wood or bioenergy (Van Slycken *et al*., 2013, Kuppens *et al*., 2015, Cundy *et al*., 2016). Stabilizing metals in the soil by using metal immobilizing soil additives and microbial inoculants (biostimulants) has beneficial effects (Kidd *et al*., 2015). Chelators can be added to the soil to increase the bioavailability of the plant-available fraction, but some chelators can also promote the mobility of the harmful metals, giving rise to leaching and contamination of surrounding soils, ground and surface waters (Sessitsch *et al*., 2013).

Phytovolatilization can also occur with some metals, in particular in the case of mercury and selenium. In the case of selenium, plants are capable of taking up, sequestrating selenium and converting the inorganic selenium into volatile organic, non-harmful components that can be volatilized without risk (Banuelos *et al*., 2002). However, for mercury this is not applicable since in this case the volatile compounds formed by naturally existing plants are toxic. The studies on this subject therefore focus on the genetic modification of the plants in question in order to achieve the desired effect (Meagher *et al*., 2007).

Phytohydraulics can also be used to contain or remediate groundwater contaminated with heavy metals. Trees can extract low concentrations of metals that are essential nutrients, while hyperaccumulators can absorb and concentrate certain metals up to 100 to 1,000 times more than the concentrations in the soil. The higher concentrations of metals in the leaves of hyperaccumulators makes them less popular for consumption by herbivores and thus gives an additional advantage to these plants that survive in these difficult soils. Phytotechnology applications for some of the metals are explained in more detail below.

Arsenic

Arsenic contaminated soils and groundwater have already been successfully remediated by means of phytoextraction. Some ferns such as the Chinese fern (*Pteris vittata*) can hyperaccumulate arsenic efficiently (Ma *et al*., 2011). These ferns grow in areas with a mild climate and have roots that can reach about 30 cm deep into the soil, depending on the soil texture and arsenic concentrations (Lampis *et al*., 2015). Phytoextraction of arsenic is applicable for large and small contaminated sites. At certain sites, hyper-accumulating ferns (such as Chinese fern (*Pteris vittata*) and gold fern (*Pitygramm calomelanos*) can accumulate more than 2% arsenic in their biomass (Gonzaga *et al*., 2006). While *P. vittata* is considered a hyperaccumulator of arsenic, the plant also converts arsenate to arsenite (a very toxic form of arsenic), this should certainly be taken into account when working with these plants. Birches inoculated with good endophytic bacteria can also accumulate arsenic in the aboveground parts (Mesa *et al*., 2017). The aboveground plant parts can be harvested for recycling. If recycling is possible, arsenic can be recovered from biomass in percentages greater than 70% by liquid extraction, which can then be used in industrial applications.

Cadmium

Phytoextraction of cadmium from contaminated soils is generally a fairly slow process. In the first place, cadmium-hyperaccumulating plants have only a small biomass and slow growth rate. Secondly, the uptake of cadmium by high biomass-producing plants such as short-rotation willow (*Salix spp.*) and poplar (*Populus spp.*) occurs slowly due to low bioavailability, slow uptake and growth-limiting factors. Recent study results of the cadmium-contaminated soils in the Noorder Kempen (Belgium) have shown that a good selection of the willow clone type can achieve an increase in metal concentrations in the strain of more than 74% for cadmium and 91% for zinc in comparison with other willow clones (Janssen *et al*., 2015). Furthermore, it was shown that by inoculation of the trees with the bacterium *Rahnella* sp. the extraction efficiency of cadmium increased due to an increase in twig biomass. Why some clones do better than others needs further study. A study from Canada showed that in addition to characteristics for high biomass and rapid growth, the success of a certain clone at a certain site also depends very much on which rhizosphere microorganisms the plants associate (Bell *et al*., 2015).

A comparative study of willow clones on a metal contaminated soil showed that the dominance of certain fungi (ectomycorrhiza) in the root zone was linked to a higher Zn accumulation and therefore cultivar fungus specificity can be crucial to explain metal accumulation. Another study comparing the bacterial communities of rapeseed (*Brassica napus*) growing on the cadmium-polluted soil in Lommel and a non-polluted soil in Alken, showed that more metal-tolerant bacteria were present in the contaminated soil, more were able to release phosphate into the soil, produce the plant hormone auxin and reduce plant stress hormones, indicating that the bacteria help the plants to survive in contaminated soils through various mechanisms (Croes *et al*., 2013). A technical-economic evaluation of phytoextraction showed that fast pyrolysis of short rotation forest with valorization of the biochar to activated carbon and the oil for bioenergy can be economically advantageous (Kuppens *et al*., 2015).

Chromium

Despite the fact that no plants are known that can hyperaccumulate chromium, studies have shown that certain plant species can be used to absorb chromium contamination from soil, surface or groundwater through phytoextraction or phytostabilization (Pulford *et al*., 2001). For example, willow (*Salix spp.*) and birch (*Betula spp.*) can absorb chromium from the groundwater, but the chromium remains mainly in the roots (Pulford *et al*., 2001). The most important findings in chromium-phytoremediation is the bioreduction of Cr (VI) to Cr (III) by the plants and microorganisms. Stinging lye (*Salsola kali*) a plant that occurs along the beach and in the dunes can accumulate chromium III, which shows that this plant can possibly also be considered for phytoextraction of chromium from the soil (Gardea-Torresdey *et al*., 2005).

Copper

Field studies have shown that willows can accumulate copper and are therefore suitable for copper-phytoextraction (Mleczek *et al*., 2013). Soil additives such as phosphate can also increase copper uptake as shown in studies with Indian mustard (*Brassica juncea*), and these can also be further investigated for phytotechnology applications (Fang *et al*., 2012).

Lead

The use of soil additives and plants such as water lilies (*Nymphaeaceae*) has proven to be effective in stabilizing lead in the soil (Lin *et al*., 2009). Because the bioavailability of lead is very low in soils, phytoextraction of lead is not possible.

The use of chelators has often been investigated to make lead more bioavailable, but therein lies the danger of overmobilizing it and causing leaching of chelated lead complexes into surface and groundwater, faster than it can be taken-up by plants. Certain fungi that occur in the root zone of specific grasses, among others, can mineralize lead into chloropyromorphite, the most stable lead metal that exists (Rhee *et al*., 2012). This conversion is irreversible and ensures that the lead present is no longer bioavailable. In this way, phytostabilization of soils contaminated with lead is possible.

Nickel

There are reports of successful remediation of nickel contaminated sites by phytoextraction by means of hyper-accumulators of the Alsem genera (*Alyssum spp.*) of the mustard family (Mengoni *et al*., 2004, Cabello-Conejo *et al*., 2014). In addition, *Alyssum* species have also been used for the phytomining of nickel, that is, extracting nickel from the plant by drying and burning (Chaney *et al*., 2007).

Selenium

Soil, sediment and surface water contaminated with selenium have already been successfully remediated using phytoextraction, phytostabilization and phytovolatilization, depending on which plants were used. For example, aquatic plants such as duckweed (*Lemnaoideae*) and water hyacinths (*Eichhornia* spp.) can remediate selenium in reed beds and other natural water purification basins (Pal & Rai, 2010). In addition, Indian mustard (Brassica juncea) and rapeseed (Brassica napus) are also used for the phytovolatilization of selenium, in which selenate is converted into the less toxic dimethylselenite gas that is subsequently released into the atmosphere (Pilon-Smith *et al*., 2013).

Zinc

Zinc can be accumulated by Alpine penny-cress (*Nocceae caerulescens*) and other metal hyperaccumulating *Nocceae* and *Arabidopsis* species, but due to low biomass and slow growth, the phytoextraction of zinc is not efficient enough (Lodewyckx *et al*., 2002). In addition to zinc contamination, copper is usually present which slows down the growth of the plants and thus also the uptake of zinc (Lombi *et al*., 2001). Phytic extraction of zinc by short rotation woody crops has also been applied with results highly dependent on the selected clone and the environment-specific characteristics such as soil fungi, zinc concentration and pH (Bell *et al*., 2015).

Radionuclides

Phytoextraction is also possible for the remediation of soil and water contaminated with radioactive elements. For example, sunflowers can remove uranium, caesium and strontium from hydroponics (Lee & Yang, 2010). In addition, plants can also absorb caesium and strontium from contaminated soils (Fuhrmann *et al*., 2002). Soil additives can also increase the plant uptake of radionuclides. Wild Sorgo (*Sorghum halpense*) planted in soil enriched with chicken manure taken-up larger amounts of caesium and strontium than other plant species in soils enriched with chicken manure (Entry *et al*., 2001).

Cyanides

Cyanides can be present in soil and groundwater as free cyanides or cyanide salts, as cyanates or thiocyanates (OCN or SCN) or as complexes with metals such as, for example, Fe, Ni and Zn.

There are four general degradation mechanisms in the biodegradation of free cyanides and organic substances with a cyanide group: hydrolysis, oxidation, reduction and substitution/transfer reactions (Ebbs S., 2004). Various organisms use a combination of these degradation mechanisms depending on external factors such as the availability of oxygen, the pH and the concentration of cyanide.

Vascular plants have the enzymes beta-cyanoalanine synthase and beta-cyanoalanine hydrolase that break down free cyanides and convert them into the amino acid asparagine (Larsen M. *et al*., 2002). The risk of volatilization of cyanide can be neglected because the plants would die before significant concentrations are reached.

Many microorganisms and plants are capable of degrading free cyanides without significant accumulation of cyanides in the leaves and without significant volatilization. This means that phytoremediation offers possibilities for the removal of free cyanides and the management of the risks arising from cyanide contamination. Removal of cyanide contamination is also possible, but since the conversion to free cyanides is relatively slow, this will also take a long time. The possibilities of phytoremediation in cyanide contamination are therefore rather situated in the area of risk management.

Table 5: Phytotechnology matrix

Contaminant	Phytotechnology-mechanism						Application					Scale					Key results	Reference
	Phytostabilization	Rhizodegradation	Phytohydraulics	Phytoextraction	Phytodegradation	Phytovolatilization	Biofilters, reed	Capping,	Repair of marshes	Hydraul. barrier	Buffer zones, green belts	Greenhouse	Laboratory	Field	Pilot study	Large scale		
BTEX		✓	✓		✓		✓			✓	✓	✓	✓	✓	✓	✓	Poplars could efficiently clean up a BTEX groundwater plume.	(Barac *et al.*, 2009)
Chlorinated solvents		✓	✓		✓	✓	✓			✓	✓	✓	✓	✓	✓		Oak, ash and associated microorganisms clean up TCE groundwater contamination.	(Weyens *et al.*, 2009)
PCB	✓	✓	✓										✓		✓		Often difficult to solve PCB contamination with phytotechnology, rather for the residues.	(Sylvestre *et al.*, 2009) (Slater *et al.*, 2011)
Explosives	✓	✓	✓		✓					✓	✓	✓	✓				Grasses and trees present on military sites can stabilize or rhizodegrade explosive contamination (TNT, DNT).	(Thijs *et al.*, 2014a) (Thijs *et al.*, 2014b) (Rylott *et al.*, 2011)
PAH		✓	✓		✓	✓		✓	✓	✓	✓	✓	✓	✓	✓		Quite difficult to degrade, yet poplars, willow and their microbial communities do have potential.	(Bell *et al.*, 2014)
Pesticides	✓	✓			✓		✓		✓	✓	✓	✓	✓	✓	✓		Zucchini can be used to take up and degrade DDE.	(Wang *et al.*, 2004) (White *et al.*, 2003) (White *et al.*, 2006)
Mineral oil, petroleum		✓	✓		✓			✓		✓	✓	✓	✓	✓	✓		Alkanes and low molecular weight PAHs can be remediated by willows, poplars, grasses and leguminous plants.	(Gkorezis *et al.*, 2016) (Page *et al.*, 2015)
Arsenic	✓		✓	✓			✓	✓	✓	✓		✓	✓				Poplars have already been used to cap landfills.	(Ma *et al.*, 2011) (Mesa *et al.*, 2017)
Cadmium	✓			✓						✓	✓	✓	✓	✓	✓	✓	Experimental willow clones with a high biomass yield improve cadmium and zinc extraction from the soil in the stem.	(Janssen *et al.*, 2015) (Bell *et al.*, 2015) (Croes *et al.*, 2013)
Chromium	✓			✓			✓					✓	✓	✓			Willow and birch absorb chromium but it remains in the roots.	(Pulford *et al.*, 2001) (Gardea-Torresdey *et al.*, 2005)

Contaminant	Phytotechnology-mechanism						Application					Scale					Key results	Reference
	Phytostabilization	Rhizodegradation	Phytohydraulics	Phytoextraction	Phytodegradation	Phytovolatilization	Biofilters, reed	Capping,	Repair of marshes	Hydraul. barrier	Buffer zones, green belts	Greenhouse	Laboratory	Field	Pilot study	Large scale		
Copper	✓			✓			✓					✓	✓	✓			Soil additives can improve copper uptake in Indian mustard, but more field studies are needed.	(Mleczek *et al.*, 2013) (Fang *et al.*, 2012)
Nickel	✓			✓			✓					✓	✓	✓			Plants from the mustard family can accumulate nickel.	(Chaney *et al.*, 2007)
Selenium	✓			✓			✓					✓					Duckweed and water hyacinth have already been used to absorb selenium from water basins and reed beds.	(Pal & Rai, 2010)
Radionuclides	✓			✓		✓	✓					✓	✓		✓		Sunflowers can remove uranium, caesium and strontium from hydroponics. Soil additives can improve the take-up.	(Lee & Yang, 2010) (Fuhrmann *et al.*, 2002) (Entry *et al.*, 2001)
Cyanides	✓	✓	✓		✓		✓	✓		✓	✓	✓	✓	✓	✓		Vascular plants are capable of breaking down free cyanides. Uptake of Berlin blue can be in the form of colloidal Berlin blue, hexacyanoferrates, hydrogen cyanide or free cyanide ions. There is no accumulation of cyanide in the leaves and hardly any or no volatilization occurs.	(Dimitrova *et al.,* 2015) (Ebbs, 2004) (Ebbs *et al.*, 2003) (Larsen *et al.*, 2002) (Trapp *et al.*, 2003)
Nutrients	✓	✓	✓				✓	✓		✓	✓	✓	✓	✓	✓		A soil with high biodiversity increased the yield of corn by 20% and significantly reduced the leaching of nitrate and phosphate to the water.	(Garnier *et al.*, 2016) (Bender & van der Heijden, 2015)

✓ means that the application has already been carried out experimentally. No ✓ does not mean that it is impossible but that relevant experiments are not yet available.

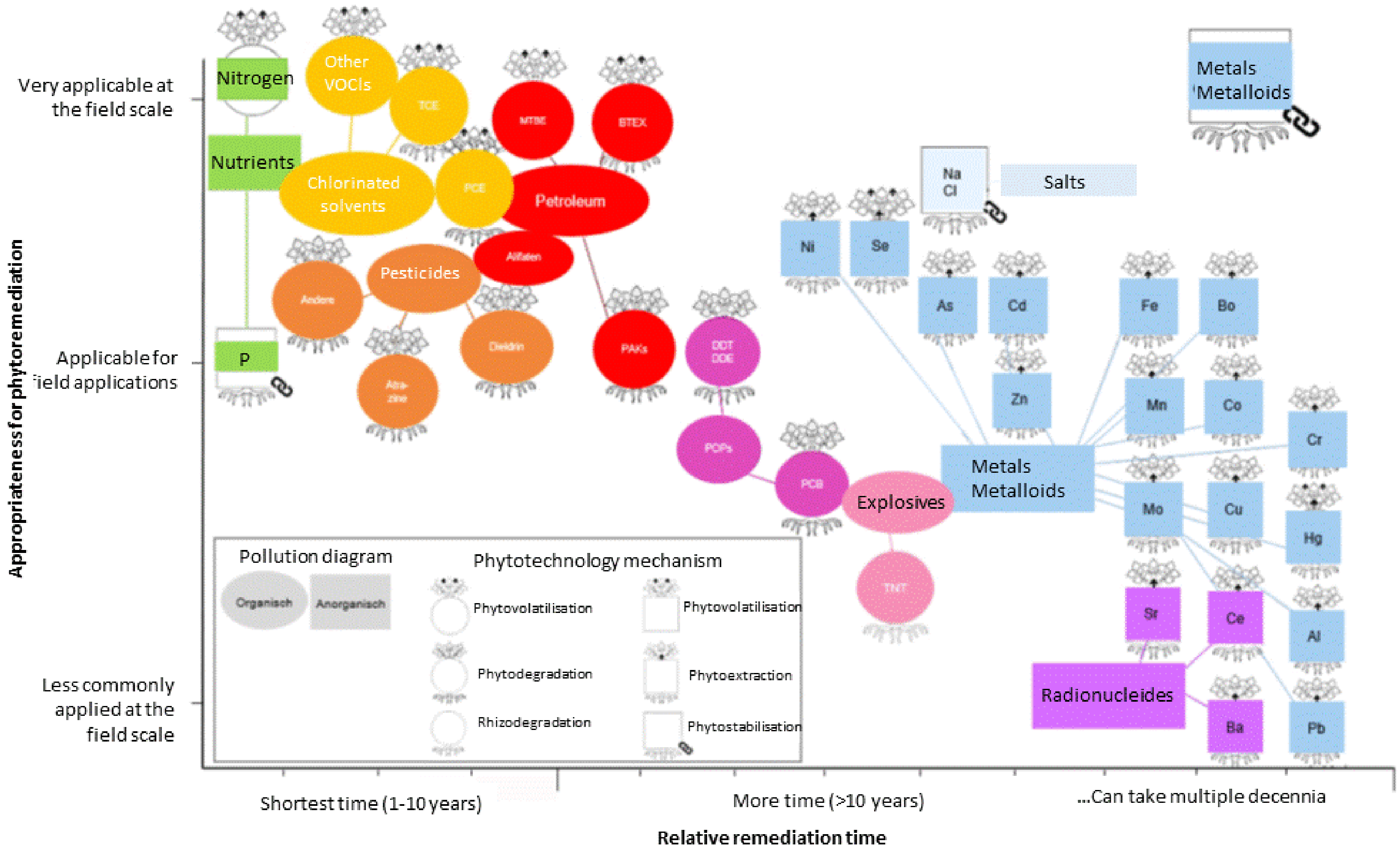


Figure 8: Overview of the phytoremediation potential of some contaminants and associated phytoremediation mechanism. Adapted from "PHYTO, Principles and resources for site remediation and landscape design," by Kate Kennen and Niall Kirkwood, 2015. Adjustments are based on information from field studies (up to 2019) and may change in subsequent editions as more remediation is performed.

2.4 PLANT SPECIES

Numerous publications can be found in the literature on phytoremediation experiments with an enormous diversity of plant species. It is of great importance to select those plant species relevant for application in Flanders from this oversupply of data. In addition to the scientific literature, a number of userfriendly databases are also available which can be used to search for plant species that have already been used for certain phytoremediation applications.

How do you start the plant selection process where you want a suitable plant species with the right characteristics for growth under specific site conditions and that can achieve the objectives of phytoremediation? There are different starting points for making the right plant selection. A few generalities in the plant selection process are explained briefly below.

Root system type

A fibrous root system has very many fine roots dispersed in the soil and will therefore, due to its large root surface, create a very large contact zone with the soil. Mycorrhiza fungi that live around the roots of the plants can increase the absorption area by up to 1000 times, whereby the ability of plants to absorb nutrients and contaminants increases. High concentrations of contaminants can kill mycorrhiza. It is therefore important to work with contamination tolerant mycorrhiza or to take additional measures such as inoculation with bacteria (mycorrhiza helper bacteria) to promote mycorrhiza growth. Many grasses such as *Festuca* sp. (tall fescue) have a fibrous root system and have been extensively colonized with arbuscular mycorrhiza.

Root depth

The root depth differs from plant species to plant species and can also vary within one species depending on the environmental conditions such as water depth, soil moisture content, soil structure, density, soil fertility, etc. Most plant roots are present at a low to moderate depth, smaller number of roots at greater depth.

Studies show that after one growing season, 75% of the underground biomass of willow and poplar is within a radius of 1.5 metres (Phillips *et al*., 2014). Compared to poplar, willows develop more and finer roots that occupy a larger volume of soil. Poplars have a less branched root system, but develop thicker roots that penetrate more easily into more compact soils. Between 30% and 50% of the total root length is less than one metre from the trunk. The class of roots with a thickness of 1.0 mm to 2.0 mm and 2.1 mm to 5.0 mm makes up 75% of the total root length with more than 60% going to the finest category that makes up only 20% to 40% of the root biomass. The maximum root depth is linked to the presence of sinkers. Sinkers are roots that develop vertically downwards from the side roots. These sinkers often have a diameter of 10 mm to 20 mm. Underground barriers such as the water table or less permeable layers prevent the deeper growth of roots. After one growing season, the roots of willow and poplar can reach a length of more than 5 metres.

The depth of the contamination should not exceed the depth of the root zone. Exceptions are the cases in which contaminated water is pumped up and made available to plant roots by f.e irrigation. The introduction of microorganisms and plant enzymes deeper into the soil is usually not as efficient because the ecological conditions are so different. Some leguminous plants such as alfalfa can root up to 9 meters deep, some grasses with a fibrous root system then root to 3 metres deep, phreatophytic trees (poplar, willow) can root up to 24 metres deep. Other plants such as rapeseed and sunflowers usually only root to a depth of 30 cm. Due to the degree of contamination or the structure of the subsoil, these maximum root depths are often not achieved at the site to be remediated. Usually 6 metres are taken into account for trees and other non-woody plants, while shrubs usually root 1 to 2 metres deep. Phytoremediation of contaminated groundwater is possible by using phreatophytes (poplar, willow) if the groundwater is at a maximum depth of 10 metres.

Growth rate

The growth rate of a plant has a direct influence on the duration of remediation. For rhizodegradation, rhizofiltration and phytostabilization, it is important to have rapid growth in terms of root depth, density, volume, area ratio and lateral expansion. For phytodegradation, phytoextraction, phytovolatilization and phytohydraulics, the growth rate of the aboveground biomass is, in turn, advantageous.

A larger root mass and aboveground biomass are important for the accumulation of larger quantities of metals, for a higher water transpiration, for a higher assimilation and metabolism of organic contaminants, or for a higher production of enzymes and more niches for degrading microorganisms.

Metal hyperaccumulators usually have only a low biomass. That is why in many cases it is more interesting to choose plants that have a larger biomass so that the total amount of metals that can be removed is larger. Poplars have often been used in phytoremediation processes in Flanders.

Transpiration rate

The transpiration rate of vegetation is important for those phytotechnologies that include contaminant uptake and hydraulic control. This depends to a large extent on the plant species, age, mass, size, leaf area, stage of growth and seasonal effects. For poplars, the transpiration rate is estimated at around 100 litres per day for a barely five-year-old tree, while up to 200 litres of water per day can be transpired through 1 willow tree.

Seed and origin of the plant

The origin of the plants is important to take into account in remediation projects:

- (i) Are the plants/seeds available locally? It is usually beneficial to work with plants/seeds specific to the region and adapted to the climate conditions. This must certainly be checked with the supplier.
- (ii) Can the plants/seeds be delivered when needed?
- (iii) Are there transport or import restrictions?
- (iv) Can the supplier provide information about the growth and care of the plants (pruning, etc.)?

Allelopathy

Allelopathy refers to the inhibition of the growth of one plant species by the presence of substances produced by another plant species. This is especially important if you want to use mixed plantations with different species. Allelopathy can also occur due to plant residues mixed under the soil e.g. carrot, stem and leaf residues of rape seed can reduce the growth of corn, wheat and barley.

Local plant species

Contaminated sites can also be naturally overgrown. It is important to check which plant species are present that can survive in such circumstances. Some of them may already degrade the contamination efficiently. Because the vegetation has been present for a long time, compared to the time that is usually intended for phytoremediation field studies, it can be an advantage for researcher and remediation expert. It remains to be considered whether extra stimulation, e.g. by inoculation with microorganisms, is necessary. The efficiency of these plants must be confirmed by greenhouse experiments and, if necessary, by a pilot experiment in the field (see also 3.4).

Plants used in phytoremediation

Currently, the plants most used and studied for phytoremediation in Flanders are: Poplar (*Populus* spp.), Willow (*Salix* spp.), Maple (*Acer* spp.), grasses (*Festuca* spp., *Agrostis* spp., *Molinia caerulea*), leguminous plants (*Medicago sativa*, *Lotus* spp., *Trifolium* spp.), agricultural and horticultural crops (*Zea mais*, *Brassica juncea*, *Heliantus annuus*, *Triticum* spp. *Cucurbita* spp.), metal hyperaccumulators (*Nocceaei caerulescens*) and aquatic plants (*Phragmites* spp., *Thypha* spp.).

Appendix 4 provides an overview of which native plants are used the most for which contaminants and which type of phytoremediation has been used successfully, which facilitates the search for plant species relevant to Flanders.

In the online database phytotechnology project profiles, US-EPA, worldwide phytoremediation cases are collected. This web-based database contains various phytoremediation projects that can be searched by vegetation type, phytotechnology, contaminant, and gives a summary of all found phytoremediation projects: Phytotechnology database.

However, it is not the case that only the plants that can currently be found in the database can be used for phytoremediation. Naturally occurring plant species that can grow under our climate conditions can be consulted on the website of the Agentschap Natuur en Bos (Agency for Nature and Forests) (ANB native tree list). In principle a large number of plants are eligible (preferably native) with a favourable growth rate and tolerance for the contamination and which can help to achieve the remediation objective.

In summary, the following plant characteristics are optimal for the different phytotechnology mechanisms:
- Rhizofiltration and phytostabilization: plants can remove metals, no translocation of metals from the roots to the shoot, fast growing root system;
- Phytoextraction: plant tolerates, translocates and accumulates high concentrations of metals in the harvestable aboveground parts (stem and leaves). High growth rate and biomass production;
- Rhizodegradation: plant excretes many enzymes and should not absorb the contaminant, suitable root growth (depth and extent), possibility to associate with a diverse and efficient microbiome;
- Phytodegradation: plant has the ability to absorb the contaminant, degradation products are non-toxic;
- Phytovolatilization: possibility to absorb and volatilize the contaminant;
- Phytohydraulics: possibility of keeping the contaminant on site by influencing the groundwater depth, flow and direction.

2.5 DETERMINING TERRAIN CHARACTERISTICS

Like all remediation techniques, phytotechnology is also highly dependent on environmental conditions such as soil type, pH, cation exchange capacity, organic matter content, presence of impenetrable layers, the depth and flow rate of the groundwater and the climate. All these factors will strongly influence the application of a phytotechnology as well as its velocity and efficiency. It is therefore crucial to clearly examine and describe the site as early as possible in the remediation process in order to determine how phytoremediation can contribute to the remediation of the contamination. This evaluation is described in more detail in 3.1 (first screening for feasibility of phytoremediation).

The most important terrain characteristics that determine the feasibility of phytoremediation are described below.

Contaminated medium (soil, sediment, (ground) water)

For phytoremediation, the contamination must be accessible to the plants. This means that the contamination cannot be located too deep in the subsoil. But if trees whose roots penetrate fairly deeply into the soil (e.g. poplars) are used, it is also possible to remediate (part of) the groundwater (to a depth of at least 10 metres). If the depth of the contaminants present presents a problem, you can choose to excavate the contaminated soils and place them in soil piles. These piles can then be sown or planted. The plants then provide oxygen and nutrients for the degradation of the contaminants (eco-piling).

It is also possible to pump up contaminated groundwater at a greater depth and then infiltrate it into the root zone for phytoremediation.

Contamination

Phytotechnologies for the remediation of metal contaminated soils take a lot of time because the plants must first develop a large root system and large biomass to absorb the metals most efficiently. The only rapid technology for the remediation of metal contaminated soils is excavation.

In contrast, for organic contamination such as BTEX, gasoline and kerosene, trees and their associated microorganisms can do particularly well to degrade the contamination (Barac *et al*., 2004; Balseiro-Romero *et al*., 2017, Yousaf *et al*., 2010, Yousaf *et al*., 2011, Page *et al*., 2015). For diesel and light fuel oil, it was demonstrated that degradation can occur under laboratory conditions. The remediation period and % degradability strongly depend on the specific composition of the mineral oil (see § 2.3), so a good oil characterization is necessary, in addition to good soil characterization.

Phytoremediation can in general only be applied to soils that allow for plant growth, i.e. most soils. On sites where too high concentrations of contaminants are present that prevent plant growth, phytoremediation may be possible in combination with one or more other remediation techniques.

Vegetation present

If vegetation is already present at the site, this can be used as the first indicator for phytotoxicity and for the estimation of other growing conditions.

External factors

Since phytoremediation is an in situ technology, there must be sufficient free space on the contaminated site. Furthermore, an area can still prove to be unsuitable due to obstructions such as underground pipes, high voltage, etc. Moreover, it is not only important that the available space is sufficiently large, the location of the available space must also meet a number of conditions. Phytoremediation can be applied either at the site of the contamination itself or at a (limited distance) downstream of the contamination. The fact that phytoremediation can be deployed at the site of the contamination, which means that no soil transport is required and that hardly any energy input is required, makes phytoremediation a sustainable remediation technique in comparison with 'traditional' remediation techniques (see 2.6).

After assessing the site characteristics to determine whether phytoremediation is possible at the site, it is important to choose the appropriate phytotechnology type together with the correct plant species (3.2 and 3.3).

2.6 ADDED VALUE WITH REGARD TO SUSTAINABILITY, QUALITY OF LIFE AND BIODIVERSITY

In addition to remediation of soil contamination, phytoremediation offers added value in terms of sustainability, quality of life and biodiversity. This section provides a brief explanation of the added value as summarized in **Table 6**.

Table 6: Phytoremediation added value

No.	Theme	Explanation of added value
	1. Sustainability	
1.1.	Consumption of materials and energy	Compared with the use of "traditional" remediation techniques, the ecological footprint of phytoremediation is considerably smaller in most cases. The need for energy and materials is particularly small when applying phytoremediation.
1.2.	CO_2sequestration	The use of sunlight versus conventional energy and the conversion of CO_2 into biomass. The biomass accumulated (above and below ground) forms an important carbon supply (sink).
1.3.	Bioenergy	Energy from biomass has three forms of energetic valorisation: heat or cooling source, electricity production and finally biofuel (liquid or gaseous). The use of bioenergy results in reduced CO_2 emissions, climate control and a reduction in fossil fuels.
1.4.	Storage of greenhouse gasses	Plants contribute to the storage of greenhouse gases (water vapour, CO_2, CH_4 and N_2O) whereby they are exchanged with the atmosphere.
	2.Biodiversity	
2.1.	Soil repair	Phytotechnologies ensure soil recovery. Phytotechnologies can then be used in combination with other soil repair projects and objectives, such as a green roof or ecological upgrade.
2.2.	Soil structure, fertility and erosion	Soil structure and fertility are not adversely affected and even improve. The plant then forms a buffer against erosion.
2.3.	Soil life and microbial diversity	The rooting and permanence of cultivation (up to 20 years) produces an intensification of soil life.
2.4.	Soil quality	Soil generates numerous ecosystem services: (i) supporting (soil formation, food cycle, habitat), (ii) regulating (regulating elementary cycles, C-capture, water purification and storage; adsorption and transformation of contaminants), (iii) providing (raw materials and biomass), and (iv) cultural (heritage).
2.5.	Wild animals and plants	Possibility to create a new habitat or to supplement an existing one. Wild animals and plants are attracted.

	3. Environmental quality	
3.1.	Urban green infrastructure	- Plants can create a more pleasant living and working environment. They also contribute to the aesthetic character. - Phytoremediation technologies can be used, in addition to and without damage to mature trees and shrubs, and in special locations such as roadsides, paving, roofs, etc.
3.2.	Noise pollution	Plants ensure sound reduction, sound buffering and sound masking.
3.3.	Air quality	Vegetation is capable of filtering fine dust and contaminants from the air or diluting contamination plumes from highways or industrial sources. This process has a favourable effect on air quality with a positive effect on public health. On the other hand, trees in narrow "street canyons" cause a decrease in ventilation and an increase in concentrations at ground level. The effect of vegetation on air quality can therefore be both positive and negative.
3.4.	(Local) climate regulation	Vegetation emits aerosols that accept and scatter solar radiation, resulting in a reduced direct radiation, which prevents e.g. heat island effects; Because of the effects of shading, evaporation, wind stops and albedo, vegetation has an influence on the local climate. Vegetation in an urban environment is able to mitigate the microclimate in the city, by cooling during the summer and by limiting the heat losses during the winter.
3.5.	Attractive natural landscapes	The landscape is improved with stimulation of green recreation. The experience of natural landscapes is classified with the cultural ecosystem services. Landscapes can be appreciated because of their recreational, cultural-historical, aesthetic and natural-scientific value.

The use of phytotechnologies generally also means a significant **reduction in start-up and maintenance costs** of remediation and repair (compared to “standard” remediation techniques), because plants are self-sustaining (photosynthesis) and self-repairing. Moreover, it can also be used taking into account the existing plants and/or vegetation, and at specific locations such as roadsides, roofs, vertical noise barriers. Phytoremediation makes it possible to combine soil remediation with numerous other functions.

Soil remediation does not always have to be the primary goal. Certain soils are contaminated but do not have to be remediated, for example with limited contaminant concentrations, with historical contaminants that pose no risk or for residual contaminants. The **greening** of such sites can offer great added value for people and the ecosystem, not least on company sites and in the built environment. Knowledge about phytoremediation in this case contributes to increasing the chance of success of a planting or vegetation development, the reduction of soil contamination and the reduction of any risks that can arise from a plantation such as mobilization of the contamination as a result of changing soil parameters, distribution via the food chain, leaf fall, plant volatilization.

In most cases, phytoremediation creates a **win-win situation** that offers many opportunities. Well-thought-out monitoring makes it possible to follow up the process and communicate about it with numerous stakeholders.

The use of green remediation techniques such as phytoremediation can count on a **large public acceptance**. If the green fulfils additional functions (ecological functions, recreational functions, aesthetic functions, etc.), this acceptance will increase even more. Combining multiple functions is an important aspect in the elaboration of a phytoremediation project. The dynamic aspect of phytoremediation must always be taken into account. It is crucial to communicate with local residents about the different steps in the process and about the concrete added value for people and the ecosystem.

Finally, phytoremediation contributes to **ecosystem recovery** (**Figure 9**). Mitigating abiotic problems is often just one of the barriers that must be overcome in the entire process of ecosystem recovery (**Figure 9**). The other barriers such as restoring biotic interactions (e.g. inoculating N_2-fixing bacteria, re-vegetation, types of reintroduction) or improving nature management (planting density, crop rotation, pruning method, fertilizer, invasive species control) are also important and are part of the aspects of "phytotechnology" for the restoration of natural heritage, biodiversity and ecosystem functions. At the same time, we must acknowledge the long-term relationship between people and their environment and take them into account when choosing phytoremediation strategies in which a healthy relationship between people (culture) and nature must be sought.

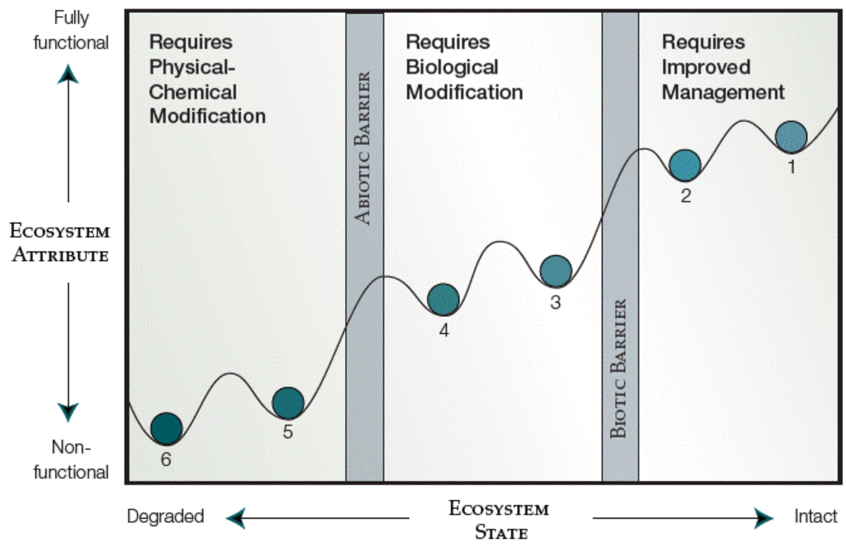


Figure 9: Conceptual model for ecosystem recovery (adapted from Whisenant 1999, and Hobbs and Harris, 2001.

2.7 REMEDIATION DURATION AND COST

2.7.1 Remediation duration

How much time is needed?

The time required for phytoremediation depends on several factors:

- the remediation objective;
- the concentrations and extent of the contamination;
- the bioavailability of the contamination;
- the depth of the contaminated zone;
- the growth rate of the plants;
- the growing season of the plant;

- the climatic conditions;
- the soil conditions;
- the accessibility of the contamination i.e. the phytoremediation can be applied in the contaminated zone (= reachable zone) or phytoremediation can only be used as a barrier (for example: when a hydraulic barrier is used, the remediation duration depends on the distance of the contamination to the hydraulic barrier and the spreading rate of the contamination to the hydraulic barrier).

These factors are site specific. It may also be necessary to replace plants if they were damaged by extreme weather conditions, diseases and animals. This can therefore extend the remediation period.

Depending on the remediation objective and therefore also the chosen phytotechnology mechanism, the remediation duration will vary. On the one hand, the remediation period will be short if phytostabilization is chosen. The remediation objective can then already be achieved within the year. On the other hand, the remediation period will be long in the case of phytoextraction of metal contaminated soils. In this case the remediation will often take several decades. For the remediation of organic contaminants by means of phytotechnology, the remediation duration will, however, be between these two extremes and will often be comparable to the remediation duration of the in situ alternatives (such as biosparging, etc.).

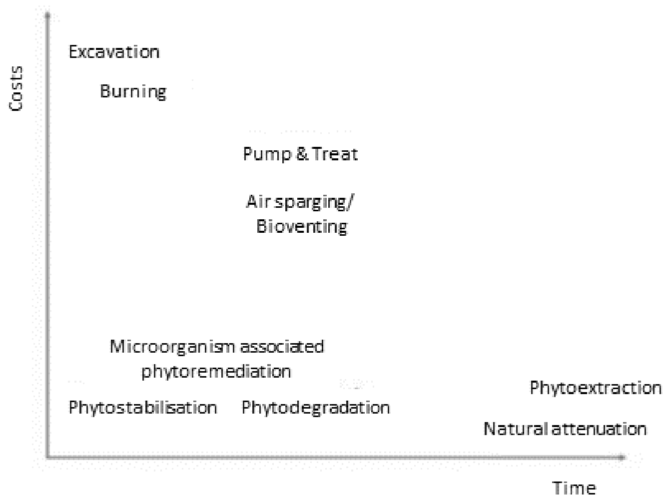


Figure 10: Phytotechnologies - costs versus remediation duration (Reynolds, 2011).

2.7.2 Cost

The cost estimate for remediation with phytoremediation takes into account four main categories (see also chapter 4): (1) Design, (2) Organization (3) Maintenance and (4) Monitoring the efficiency and result of remediation (= sampling and analysis).

(1) Costs for the design, preparation of the soil remediation project, preparation of tender specifications include feasibility studies, plant selection and the associated engineering costs for the preparation of the soil remediation project and specifications for the execution of the works. Greenhouse experiments or pilot tests may be needed to determine which plants to use and to assess the possibility of phytoremediation as a treatment option for the site. In principle, these must be carried out prior to the preparation of a soil remediation project. The preparation of specifications for the execution of contracting works can be kept fairly limited in phytoremediation: type of plant, planting plan, drainage pipes, etc. as well as the need to remove any land barriers at the location.

(2) Installation costs include site preparation, soil preparation, materials and labour. To prepare the site, it may need to be cleared, levelled or fenced. Soil preparation can relate to pH adjustment, nutrient supply or processing in general. Site and soil preparation may in some cases require work and/or materials, including heavy equipment, organic substances, irrigation systems, plant material (including a 10-20% surplus for replanting needs (ITRC, 2004)) and plant protection materials for plants.

(3) Maintenance costs include the installation of monitoring wells, possible costs of power sources (in case a pump is needed for recirculation), irrigation, pruning and care of the plants/trees and labour are included. Specific requirements for phytoremediation management are described in section 4.4 of this document.

(4) Follow-up of the efficiency and result of remediation through sampling and analysis of soil or groundwater. These costs can dominate the total costs of the project due to the time required for monitoring and the required amount of data. The costs mainly include sampling time (carrying out control drilling and groundwater sampling) and laboratory costs for analysing the samples. Data collected during sampling and analysis are crucial for thorough documentation of the site progress and the performance of phytoremediation as a new technology.

The costs associated with these four categories are relatively small compared to traditional “standard” remediation techniques. This is especially the case in the operation and maintenance phase, where the primary factor for cost reduction is the energy source for the control systems. Traditional systems use electric power, at considerable costs, to pump water, for example, while phytoremediation systems use free solar energy. Individual sites vary in costs regardless of the technology used. In general, phytoremediation is a cheap alternative to traditional methods, as can be seen in the cost estimates of **Table 7**.

Table 7: Total remediation project costs (Green & Hoffnagle, 2004)

Problem	Standard remediation	Phytoremediation and passive technologies	Cost (EUR) standard remediation	Cost (EUR) phytoremediation	Project costs saved	Reference
Metals in Soil (Cd, Cu, etc.)	Flushing/ Vitrification	Phytostabilization/ extraction harvest and disposal	60-170 EUR/ton 240-400 EUR/ton	20-80 EUR/ton	45-50 %	Blaylock *et al*., 1997
Solvents in groundwater, 1 ha	Pump and treat	Phytodegradation and hydraulic control	700 000 annual running cost	200 000 installation and initial maintenance	50 % cost saving from the third year	Solvent recovery Systems of New England
TPH in groundwater, 6 m, 0.4 ha	Pump and treat	Phytodegradation and hydraulic control	535 800	201 126	62 %	Gatliff, E., 1994
TPH in Soil, 0.4 ha	Excavation and disposal, combustion	*In situ* degradation	500 000	50 000 - 100 000	80-90 %	Drake 1997
Kerosene, diesel, benzene, BTEX in groundwater (567 000-756 000 L)	Pump and treat	Passive oil float layer removal and phytoremediation	Estimated to be 1 million	326 985	67 %	Nichols *et al*., 2014
PCE in the groundwater	Pump and treat	Phytodegradation and hydraulic control	1.89 -1.12 EUR/1000L	0.43 EUR/1000L	> 50 %	Schnoor 2002

For some phytoremediation technologies, hypothetical cost comparisons have already been projected in the past. **Table 7** shows estimates based on work carried out on a laboratory and pilot scale. They try to reflect the projected total project costs.

The costs for phytoremediation are highly dependent on the mechanism that is applied and are project and location dependent.

Based on the results of the above cost estimates for the total project costs for the implementation of remediation, as well as the execution of an extensive literature study regarding the remediation costs for phytotechnology projects, **Table 8** - Phytotechnologies: Costs related to remediation duration were further supplemented.

This table also contains the data from the project files that form part of this code of good practice, as well as the data from relevant projects (full-scale and for which costs are available) from the online phytotechnology database (US EPA).

Briefly summarized:

- **Phytoextraction**: the remediation costs depend on many factors and can therefore vary from a few euros per square meter to almost €500/m^2.
- **Phytostabilization**: a comparison of 6 phytostabilization projects teaches us that the costs can vary from a few euros per square meter to €65/m^2.
- **Phytodegradation**: Studies of eight projects where phytodegradation was applied for the remediation of a site with an organic contamination show that the costs are mainly between ten euros and around €65/m^2. Here too, an outlier of €323/m^2 was recorded.
- **Hydraulic control**: Based on 12 projects in which phytohydraulics plays a major role, it can be decided that the costs can vary from a few euros to almost €100/m^2, with an outlier of €862/m^2.

Table 8: Phytotechnologies – cost versus remediation duration (Reynolds, 2011)

Contaminant	Rhizodegradatie	Fytodegradatie	Fytovolatilisatie	Fytohydraulica	Fytoextractie	constructed wetland	Fytostabilisatie	Microbe geassisteerde fytodegradatie	Doel/Design	Grassen	Populier	Wilg	Esdoorn	Ceder	Spar	Eucalyptus	Tamarisk	Ruwe berk	Witte els	Varens	Duinriet	Klaver	Wonderboom	Fungi	Zonnebloem	Mosterdplant	Mais	Courgette	Oppervlakte (ha)	Diepte (m)	Saneringsduur (jaar)	Tot Kosten	Kosten in €/m2	Locatie	Site
Anorganisch: As				x												x	x												0,49		10	€ 78.523,75	16,17	California	
Anorganisch: As					x															x									0,004		#	€ 3.489,94	86,24	Texas	
Anorganisch: As					x															x									0,40		#	€ 1.983.187,19	490,06	Virginia	
Anorganisch: As, Pb, Zn					x				(lange termijn) remediatie			x																	20,00	0,50	2	€ 90.000,00	0,45	Spanje	3
Anorganisch: Cd, Cu, Pb, …					x		x							x		x													10,93		3	€ 134.362,87	1,23	Texas	
Anorganisch: Cd, Cu, Pb, Zn							x		risicocontrole	x																			135,00	0,35	0	€ 1.350.000,00	1,00	Maatheide	2
Anorganisch: Cd, Pb, Nitraat, Sulfaat							x			x	x																		6,88		11	€ 226.846,40	3,30	South Carolina	
Anorganisch: Cd, Pb, Zn					x																		x		x	x	x		0,50		9	€ 47.986,74	9,60	Poland	
Anorganisch: Cd, Pb, Zn					x				(lange termijn) remediatie			x																	10,00	0,30	8	€ 45.000,00	0,45	Lommel	1
Anorganisch: Cu, Zn							x		Risicocontrole	x	x	x																	10,00	0,35	0	€ 140.000,00	1,40	Frankrijk	5
Anorganisch: Ni				x					Hydraulische barrière		x	x																	0,36	6,00	5	€ 0,00	0,00	Harelbeke	4
Gemengd: As, Pb, Zn, VOCl	x	x		x	x		x			x	x	x																	1,62		5	€ 1.046.983,38	64,68	Illinois	
Gemengd: Cr, Olie, VOCl	x		x	x						x	x																		1,21		8	€ 61.074,03	5,03	Florida	
Organisch: BTEX		x		x					Remediatie pluim		x																		12,50	9,00	4	€ 308.837,50	2,47	Genk	7
Organisch: BTEX		x		x							x	x	x																0,24		4	€ 43.188,06	17,79	Ohio	
Organisch: BTEX	x	x									x	x	x	x	x														8,09		3	€ 26.174.584,50	323,39	Michigan	
Organisch: BTEX				x						x	x																		4,05		2	€ 627.317,54	15,50	Ohio	
Organisch: BTEX	x			x						x	x																		0,81		8	€ 6.979.889,19	862,38	New Hampshire	
Organisch: Chlorendisch zuur				x					Risicocontrole		x													x					0,87	6,00	30	€ 52.697,00	6,06	Genk	11
Organisch: DDE								x	Remediatie																			x	-	0,30	1	€ 0,00		USA	10
Organisch: Olie		x		x					Remediatie kern + pluim		x	x																	0,30	7,00	8	€ 61.524,30	20,51	Genk	9
Organisch: Olie	x	x								x												x							0,09		10	€ 23.775,25	25,59	Alaska	
Organisch: Olie, diesel, BTEX	x	x		x						x	x																		0,16		4	€ 69.798,89	43,12	Wisconsin	
Organisch: Olie, diesel, BTEX	x	x								x												x							0,06		1	€ 12.781,92	22,93	Alaska	
Organisch: VOCl				x				x	Remediatie pluim		x																		2,00	10,00	10	€ 810.000,00	40,50	Vlaanderen	8

3 PROCEDURE FOR FEASIBILITY TESTING AND PREDESIGN

Before the start of a remediation project, it is important to know (1) whether remediation is required (as evidenced by a **descriptive soil survey** and associated **risk assessment**), and (2) if remediation is necessary, what the remediation objectives are: desired end point of the contamination (degradation, fixation, etc.) and desired concentrations. A suitable remediation technology can then be selected to achieve the remediation objectives.

A **feasibility analysis**, or evaluation of selected preferred remediation options, is carried out to obtain sufficient information about remediation options suitable for the contaminated site to achieve the identified objectives. The feasibility study thus forms the basis for selecting a suitable remediation option, which efficiently removes the hazardous substance(s) or minimizes its exposure to humans and the environment by fixation (stabilization) or transforming it into less harmful substances. The way in which the feasibility study is carried out and the results thereof must be included in the soil remediation project.

This chapter focuses on the **evaluation of phytoremediation as a possible remediation alternative**. In what follows, the comparison of phytoremediation with other remediation options is not explicitly made; we focus primarily on the feasibility of phytoremediation. In reality, a comparison of efficiency, cost, and duration must be performed with various other remediation alternatives. **Furthermore, phytoremediation can also be interested in combination with other remediation techniques**. A typical example of this is passive floating layer recuperation with a floating layer recuperation unit for the removal of petroleum hydrocarbons floating on groundwater, followed by phytoremediation. The combination of both passive techniques has already proven successful for remediation of a shallow contaminated aquifer at a fuel storage terminal in Elizabeth City, NC, USA (Nichols *et al*., 2014). A floating layer recovery unit removed 109.561 L of free-phase petroleum product and then the trees took over with as a result a decrease in thickness of the floating layer in the phytoremediation zone.

The process that must be followed to evaluate whether or not phytoremediation is a feasible option is shown schematically in **Figure 11**. This process also forms the basis for the design of the phytoremediation process (Chapter 4).

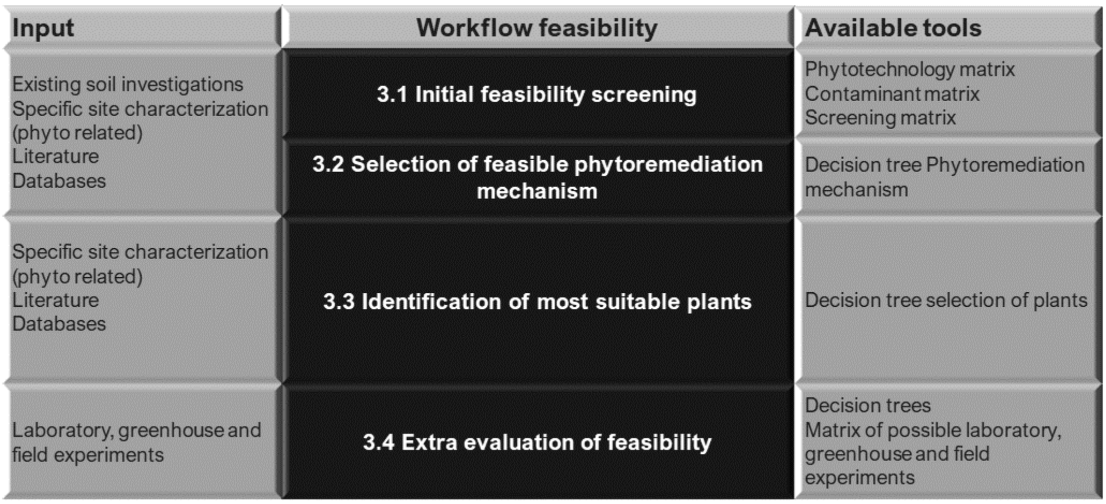


Figure 11: Workflow feasibility analysis

A first step in the feasibility analysis is a **initial screening for the feasibility of phytoremediation** (3.1) in general. Important parameters here are the characteristics of the contaminated medium (soil, sediment, water, etc.), the contamination (type and concentration), the vegetation present and site specific factors (possible obstructions and available space). Previously conducted soil investigations, the literature, available databases and an additional phyto-specific field characterization provide sufficient information to perform this initial screening. For mineral oil, oil characterization is important to estimate the effective composition (see section 2.3).

Furthermore, the phytotechnology matrix (**Table 5**), the overview of the phytoremediation potential of the various contaminants (**Figure 8**) and the screening matrix (**Table 9**) can be used as a tool in this initial screening for feasibility. This screening can lead to 3 different conclusions: (1) phytoremediation is not feasible at this site, (2) phytoremediation can be interesting for a certain part of the contamination and must be combined with another remediation technique or (3) phytoremediation can be applied for all of the contamination.

If the outcome of this screening appears to be positive (conclusion 2 or 3), the next step will determine which **phytoremediation mechanism** is the **most suitable** (**3.2**). For this the decision tree "phytoremediation mechanism" (**Figure 14**) must be followed step by step and no additional input is needed.

Once the most suitable mechanism has been determined, **the plant choice** can be made (**3.3**). Here too a decision tree has been drawn up (**Figure 15**) to facilitate the selection. The phyto-specific field characterization, the literature and available databases are sufficient here as a source of information.

If the feasibility screening is positive and the phytoremediation mechanism as well as the most suitable plant(s) is/are identified, the next step is a thorough feasibility analysis. Depending on the type of contamination and the existing experience with phytoremediation of the contamination in question, different feasibility tests may have to be carried out. Here too, a decision tree is provided that should be followed step by step to determine which feasibility tests are needed (**Figure 16**). The **feasibility** of the most suitable phytoremediation mechanism will be **thoroughly tested** (**3.4**) for 3 to 5 interesting plants selected. Furthermore, it becomes clear in which cases a switch has to be made from classical phytoremediation to microorganism assisted phytoremediation. In addition, **Table 10** provides an overview of the various laboratory and/or greenhouse experiments that may be required and an estimate of the duration and cost associated with it. The results can also be used to choose the most optimal plant.

Once this process has been completed, a preliminary design of the final full-scale remediation method is reached. The full-scale design, follow-up and monitoring are discussed in Chapter 4.

3.1 INITIAL SCREENING FOR THE FEASIBILITY OF PHYTOREMEDIATION

Depending on the application of phytoremediation, it is recommended to perform a thorough phyto-specific site characterization (**3.1.1 Phyto-specific site characterization**). Once the phyto-specific site characterization has been carried out, a first estimate can be made of the feasibility of phytoremediation based on a number of user-friendly tools (**3.1.2 Estimation of feasibility of phytoremediation**).

3.1.1 Phyto-specific site characterization

The site survey can be done by means of a tour on site. It is important here to make clear notes and sketches supplemented with the necessary measurements, so that they can be used in the design afterwards. Attention must be paid, among other things, to soil characteristics, the presence or absence of vegetation and its condition, the condition and location of existing utility pipes, buildings and pavements, the boundaries of the site, etc. During this phyto-specific site characterization it is useful to take some soil samples for additional analyses.

Performing a correct and complete phyto-specific site characterization is of great importance for the proper design and implementation of the phytoremediation project.

It is important that after this phyto-specific site characterization the information already available in former investigations can be supplemented in such a way that the parameters described below for the **contaminated medium** (**3.1.1.1**), the **contamination** (**3.1.1.2**) itself, the **vegetation present** (**3.1.1.3**) and a number of **external factors** (**3.1.1.4**) are known in sufficient detail.

3.1.1.1 Contaminated medium

Soil and sediment

For contaminated soil and sediment the main criteria are the depth and volume of the contaminants and soil characteristics such as texture, water content, nutrient content, pH and permeability, which determine whether or not the contamination is accessible to the plant.

The contaminated soil must fall within the radius of influence of the root zone of the selected plants. It should be taken into account that plants develop roots as a function of extracting water and nutrients to the extent that they need them to maintain themselves. For example, roots develop in the zones that contain the most moisture and nutrients and that offer the least resistance to root growth. These factors often limit the development of roots in a contaminated zone that is characterized by a lack of moisture and/or nutrients or that is more difficult for the roots to penetrate. Many contaminated sites consist of disturbed soils that are low in nutrients and are often highly compacted. However, this can easily be solved by a prior soil treatment, whereby the top layer (- 40 cm-mv) is ploughed and the necessary fertiliser is applied.

After prior soil treatment (if necessary), the accessibility of the contamination for the plant can be guaranteed if the following conditions are met:

1. The contamination is present between 0-8 m-bgl;
2. The contamination is no deeper than 5 m in the saturated zone;
3. Absence of compact/impenetrable layers between the roots of the plant and the contamination (such as sandstone layers).

If impenetrable/compact layers are nevertheless present, breaking them open mechanically and/or using treewells may be considered, if necessary (**Figure 12**).

(Ground) water

Natural reed beds and other biofilter installations are used for the remediation of surface and waste water (see the literature study). For groundwater, as for soil and sediment, the accessibility of the groundwater and the contaminated zone is the most important determining factor.

The depth of the water table must fall within the reach of the plant roots. However, by selecting the appropriate plant, a deeper groundwater table (e.g. -10 m) can also be achieved without problem. This concerns “phreatophytes”, being plants that always look for groundwater with their roots.

It is also important that phytoremediation usually only occurs in the upper layers of groundwater because plant roots do not pass through non contaminated groundwater to reach the deeper contaminated groundwater. The contamination must therefore not be too deep (< 5 m) under the groundwater table.

There are also seasonal fluctuations in the water table that influence the root depth.

If deeper groundwater layers still have to be remediated, one can proceed in phases, whereby first the contaminated deep groundwater is pumped up and subsequently infiltrated in the shallow zone at the level of the plant roots. Infiltration must be calculated/modelled in such a way that infiltration of contaminated groundwater does not lead to additional contamination in the shallow groundwater.

Finally, as for soil and sediment, the accessibility of the (ground) water can be impeded by impenetrable layers. The presence of hard layers, for example a clay layer in a sand package or sandstone layers, can influence the development of the roots. These hard layers can be broken through the use of tree wells or tree sleeves consisting of material impervious to the roots as depicted in **Figure 12a**. In this way root growth can be guided through these impenetrable layers to the deeper groundwater.

a) b)

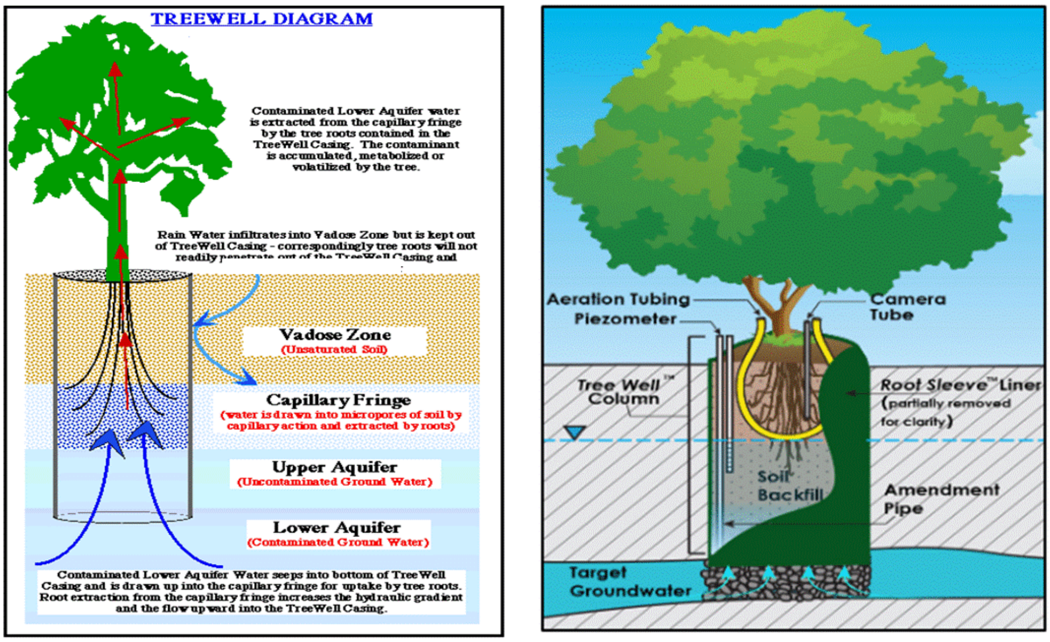


Figure 12: a) Tree-well sleeve to guide roots through harder layers and reach deeper contaminated groundwater. b) Example groundwater remediation of 1,4-dioxane with a Tree-well. Bioaugmentation of the root zone can be used to accelerate 1,4-dioxane degradation. Adapted from SiREM (https://www.siremlab.com/14-dioxane-bioremediation-update/).

Groundwater contamination with 1,4-dioxane is also a known problem in Flanders because of its use as a solvent and solvent stabilizer for 1,1,1-trichloroethane. Anaerobic iron-reducing bacteria could biodegrade 1,4-dioxane, but that happens slowly. That is why there is interest in accelerating the aerobic degradation of 1,4-dioxane. Aerobic co-metabolization and bioventing strategies are used to add oxygen to the groundwater. Bacteria such as *Pseudonocardia dioxanivorans* CB1190 (Mahendra *et al*., 2006) have special monooxygenase enzymes that allow the use of 1,4-dioxane as the only carbon and energy source.

SiREM has successfully used the Tree-well several times at 1,4-dioxane sites. The plants absorb groundwater through their roots and help provide aerobic conditions in the root zone, thereby creating ideal conditions for microbial 1,4-dioxane degradation. The accelerated root zone degradation by bacterial strains such as CB1190 also ensures that phytovolatilization of dioxane is limited.

Another factor is the groundwater velocity.
On the one hand, this will determine which dimensions of planting are required in the winter period, when the pumping capacity of the plants is negligible, to prevent the contamination spreading further under the planting. A rule of thumb that can be applied here is the following: the distance that the contamination must cover under the planting must be at least twice the distance covered by the most mobile component of the contamination or the most mobile degradation parameters per year. For example, if the velocity of the most mobile component of the contamination is 5 m/year, the planting should be 10 m wide in the direction of the flow direction. If the remediation objective concerns a hydraulic barrier, it must be ensured that the flow rate is (at least) proportional to the uptake and transpiration by the plant.

On the other hand, the groundwater velocity can also have an influence on the duration of the remediation process. This applies if the planting is installed downstream of the source of the contamination, in this case the duration of the contamination is determined by the groundwater speed.

3.1.1.2 Contamination

The type of **contamination** must also be taken into account in the feasibility analysis. For organic contaminants, the hydrophobicity, expressed in the log K_{ow} value, determines to what extent the contaminant will be taken up by the plants (a log K_{ow} between 0.5 and 3.5 means good uptake by plants, < 0.5 too hydrophilic and therefore a low uptake, > 3.5 too hydrophobic and binding in the cell wall). For metal contaminants, studies have already calculated the phytoextraction coefficients (the metals can be ordered from the easiest to the most difficult to extract: Cr^{6+}, Cd^{2+}, Ni^{2+}, Zn^{2+}, Cu^{2+}, Pb^{2+} and Cr^{3+}). Phytoremediation can also proceed differently for mixtures of metals than for individual metals, even when they are mixed with organic contaminants.

In the case of organic contamination, the degradability (biological and/or chemical) is important. If biodegradation is possible, it can be used optimally via phytoremediation, which is a major advantage.

The **concentrations of the contaminations** cannot be too high; they may not be too toxic to plants or elicit unacceptable negative effects on plant growth and yield. Hereby it is crucial that phytotoxicity is determined by the plant-available concentration of the contamination and not by the total concentrations. For example, a total concentration of 5 mg/kg ds Cd e.g. can be phytotoxic on a typical acidic sandy soil where nearly all the metals present are plant-available, while with the same total concentration of metals in a clay soil, plants experience no negative effects at the same total concentration because in that case the concentration of plant-available metals is much lower. This is due to a strong binding of the Cd to the organic material present as well as to the clay particles. Often only the total concentration is known on the basis of the results of regular soil investigations. To determine the available fraction of the total concentration, soil samples can be taken at different locations on the site during the site visit. Of these samples, both the total concentrations and the plant-available concentrations (0.01M calcium chloride extraction) must be analysed in order to make a correct comparison for the same sample. Those analyses can be easily carried out by standard recognized laboratories.

3.1.1.3 Vegetation present

In certain cases, there is **vegetation** at the contaminated site or an adjacent site. Observing root development, for example in a trench, can provide a lot of useful information. Vegetation present at the site can also give a good impression of possible species that are **tolerant** to the contamination (e.g. no yellow discoloured leaves, no or strongly reduced growth). If there is doubt as to whether or not phytotoxicity occurs with the vegetation present and which plant species are already present, an extensive inspection on site by a plant expert can provide a definitive answer. This only concerns visual observations.

3.1.1.4 External factors

In addition to the contaminated medium and the contamination itself, there is also a number of external factors that must be taken into account to estimate the feasibility of phytoremediation.

First of all, there must be sufficient **free space** at the right location. Ideally, there is sufficient space at the location of the contamination itself, otherwise planting at a limited distance downstream of the contamination can also be considered. The minimum space required can be estimated based on the velocity of the most mobile component of the contamination (see 3.1.1.1). In the case of a relatively low groundwater velocity, for example, a few rows of trees may be sufficient as an hydraulic barrier.

Once it is clear how much space is needed and where, an evaluation of potential other **obstructions** at this free space, such as high-voltage cables, other underground cables, pipes, foundations, etc. must be made.

Phytoremediation is usually considered to be an *in-situ* technology by providing vegetation in areas of contaminated groundwater or soil. Soil, however, can also be excavated and placed in a “treatment unit” where phytoremediation can then take place. The same also applies to groundwater or surface water. The water can also be pumped to a “treatment unit” where phytoremediation can take place, or it can be used as irrigation water. In short, if the site-specific characteristics are not favourable for the *in situ* application of phytoremediation, applying phytoremediation in an *ex situ* “treatment unit” or performing it in combination with other techniques may still be considered.

3.1.2 First estimate of the feasibility of phytoremediation

A whole range of tools are available to estimate whether phytoremediation can be applied.

For the purpose of a feasibility study for phytoremediation, an evaluation is made of the following parameters: contaminated medium, contamination and external factors.

Table 9: Screening matrix

Screening criteria	Advantageous	(intermediate)	Disadvantageous
Contaminated medium- soil, sediment, silt (see 3.1.1.1)			
Soil composition: Horizontal and vertical heterogeneity, Position of low permeable layers	Contamination accessible	Contamination accessible with extra measures	Contamination is not directly accessible to plants
Depth of contamination	Contamination accessible	Contamination accessible with extra measures	Contamination is not directly accessible to plants
Contaminated medium- Groundwater (see 3.1.1.1)			
Soil composition: Presence of low permeable layers	Contamination accessible	Contamination accessible with extra measures	Contamination is not directly accessible to plants
Depth of contamination	Contamination accessible	Contamination accessible with extra measures, other plant choices, etc.	**<u>Contamination not accessible</u>**
Groundwater flow rate/spread rate contamination (➔ influence on necessary space)	Slow ➔ little space needed for plants	Average	High➔ more space needed for plants
Contamination (see 3.1.1.2)			
Type of contamination	See Figure 8		
Organic components	0.5 < Log Kow < 3.5		Too hydrophilic, Too hydrophobic
Inorganic components	Depending on the remediation objective		

Contamination concentration based on visual inspection and/or bioavailability tests in a lab	Low risk of phytotoxicity	Moderate risk of phytotoxicity	High risk of phytotoxicity
Vegetation present (see 3.1.1.3)			
Vegetation present and root development	Very good growth/root development	Moderate growth/root development	Strongly reduced growth/root development
External factors (see 3.1.1.4)			
Free space	Free space available on the right location	Moderate free space available and/or not on the right location	Little free space available and/or not on the right location
Presence of aboveground structures/ obstructions (e.g. pavements)	Not present	Limited presence	Present over large area
Presence of underground structures (cables, pipes, foundations, etc.)	Not present	Limited presence	Present over large area

Conclusion Screening:

- Phytoremediation may be feasible if screening criteria were assessed primarily as beneficial/intermediary
- Phytoremediation not feasible as a sole remediation technique, if none of the screening criteria was assessed as beneficial, or if a criterion was bold underlined (definitive breakpoint)

In the literature study of this Code of Good Practice, the phytotechnology matrix (**Table 5**) and the overview figure of the phytoremediation potential (**Figure 8**) are already provided as useful tools.

- Phytotechnology matrix (**Table 5**): indicates for each category of contaminants which phytotechnology mechanism is involved, which applications have already been implemented and proved successful, the scale on which it has already been applied and a brief explanation of the main findings and references.
- Overview figure of the phytoremediation potential (**Figure 8**): Estimation of the phytoremediation potential for all contaminants, the most important criteria being the duration and the possibility of upscaling to field applications.

In addition, it is also possible to search the online phytoremediation database or in **Appendix 4** for previously implemented applications of phytoremediation of the contamination.

In addition to this rather general estimate, it is crucial to consider the site-specific characteristics in order to be able to make a feasibility estimate specifically for the contaminated site in question. The parameters that are important here have already been extensively discussed in 3.1 (phyto-specific site characterization). To facilitate interpretation of the results, the screening matrix can be used (**Table 9**).

3.2 SELECTION OF THE MOST SUITABLE PHYTOREMEDIATION MECHANISM

Once the necessary site characterization has been performed and consequently the most relevant data is available, it can be determined which phytoremediation mechanism is most suitable for the site in question. The step-by-step following of the decision tree below (**Figure 14**) will lead to the selection of the most suitable phytoremediation mechanism. In the case of biodegradable volatile contaminants, it must be guaranteed that sufficient degradation occurs so that volatilisation via the leaves, i.e. phytovolatilization, is avoided.

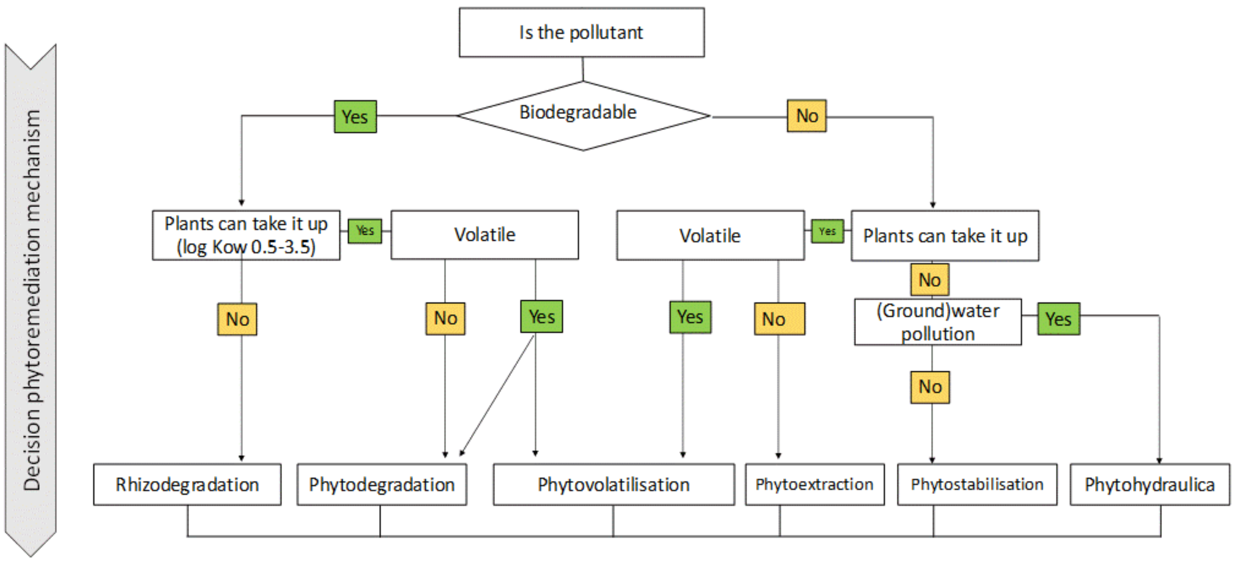


Figure 14: Phytoremediation mechanism decision tree.

3.3 IDENTIFICATION OF MOST SUITABLE PLANT(S)

For phytoremediation, it is important to choose a plant species that can achieve the remediation objective and has the appropriate growth characteristics. A first requirement is, of course, that the plant species must be adapted to the conditions of the contaminated site.

A good indication of the success of certain plant species are **earlier successful applications at similar sites**. Phytoremediation is relatively new for Flanders, but has had several field cases in the US and other countries since the early 1970s. The different databases as described in 3.1.2 can facilitate the search for earlier similar applications. In addition, a list of plants that are interesting for Flanders has been drawn up with an overview of previous successful applications (**Appendix 4**).

The decision tree below for the plant choice (**Figure 15**) can help to select the most suitable species that may be applicable.

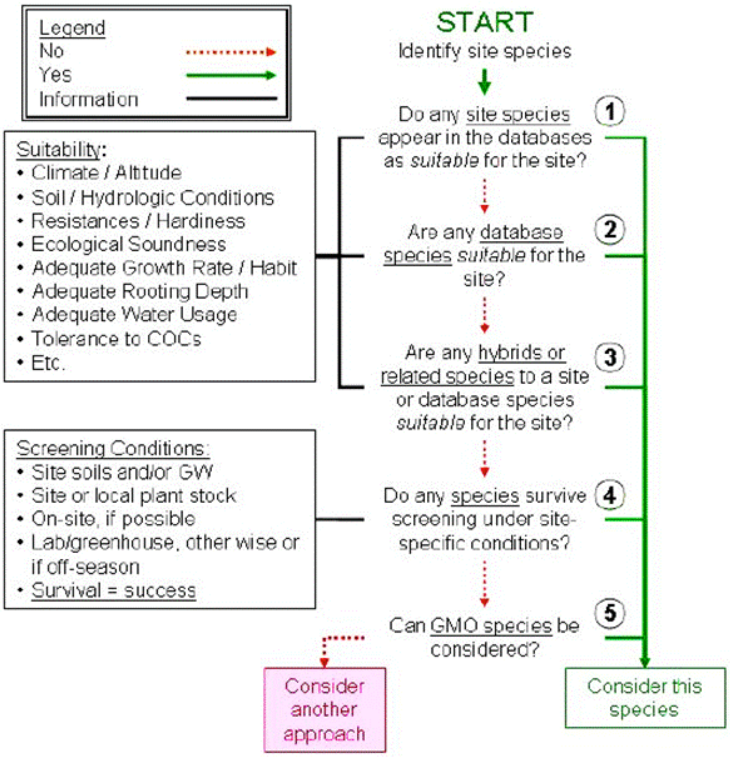


Figure 15: Decision tree for plants.

Going through the plant decision tree (**Figure 15**) assumes that the plants present at the site have been identified and starts with:

(1) species found in the phytotechnology database and currently present in the field;
(2) species found in the phytotechnology database and **Appendix 4**, and applicable, but currently not present at the site;
(3) hybrids or species related to a species identified as a potential candidate under point 1 or 2;
(4) species not found in the database or Appendix 4 but currently present at the site or surrounding area.

The applicability of a species, as discussed in the first 3 categories, is based on the species ability to grow and survive taking into account the site-specific conditions (soil characteristics, contamination, climate, etc.).

Once it is known which plant species survive at the contaminated site, there are a number of additional factors that must be taken into account.

In addition to the nature of the contaminants, the depth of the contamination is also important. With a deeper contamination, the use of deep-rooted plants will be necessary to achieve sufficient remediation effect. It is of course always possible to combine phytoremediation with other techniques to have a sufficient effect on the deeper contamination. For example, groundwater can be pumped and subsequently infiltrated into the root zone.

Furthermore, the origin of the species should be evaluated. Where possible, native species should be chosen because they are well adapted to local soil and climate conditions and their life cycle is closely linked to those of many native organisms. Studies have shown that native plants form even better associations with local degrading soil microorganisms than non-native plants (Bell *et al.*, 2014). Choosing native species also increases the ecological value of the planting. Information about **native plant species** and other characteristics can be found in the "Vademecum of Agentschap Natuur en Bos".

The outcome of this decision tree shows a list of species that may be applicable, but it certainly does not lead to a final plant selection for the site. For this, in most cases a thorough feasibility analysis based on laboratory, greenhouse and possibly field experiments are necessary (see section 3.4).

More specifically for Flanders, it can be said that for the phytoremediation of Cd, Zn, Pb, BTEX, diesel, gasoline, light fuel oil and kerosene sufficient knowledge and experience has been gained to base the further choice of the plant, after thorough terrain characterization, on phytotoxicity tests (based on greenhouse experiments) and on phytoremediation cases already available (see 2.3.1). For mineral oil, an EPK/VPK analysis or oil characterization is important to be able to estimate the composition (see section 2.3). Based on this oil characterization, it can be evaluated whether phytoremediation cases or greenhouse experiments are already available (see 2.3.1).
Additional experiments are required for the contaminants other than those listed above. These relatively inexpensive experiments can, in addition to ensuring optimal plant selection, also indicate the potential efficiency of site-specific remediation.

3.4 IN DEPTH EVALUATION OF FEASIBILITY

Depending on the selected phytoremediation mechanism, as well as the type of contamination and the available experience with relevant phytoremediation applications, certain feasibility analyses may have to be carried out. After all, there are many factors that determine the success of phytoremediation at a certain site, including the concentration of the contaminant, the availability of nutrients, the temperature, precipitation, aesthetic considerations and the presence of growth-limiting factors (contaminants, pH, etc.). The desired degree of remediation and duration must also be taken into account. All these elements need to be evaluated in advance before spending a lot of time and money on full-scale remediation.

To know which analyses must be carried out, it is sufficient to follow the decision tree for feasibility analyses (**Figure 16**) (3.4.1). This decision tree also clearly indicates in which cases it is necessary to switch from classical phytoremediation to a microorganism assisted phytoremediation. By inoculating microorganisms, certain microorganisms in the soil, rhizosphere and/or in the plant can be enriched. The function of the bacteria can be different: bacteria that ensure improved plant growth, or a higher uptake of the contaminant, or bacteria that can achieve a complete degradation of the organic contaminant. In 3.4.2 an overview is given of which laboratory experiments and greenhouse experiments should be performed for the various feasibility analyses. These experiments are **small-scale tests** with limited duration and should be performed prior to the remediation project. The type of contamination (metals vs organic contamination) as well as the chosen remediation mechanism (phytostabilization, phytoextraction, phytodegradation, rhizodegradation, phytohydraulics, phytovolatilization) determine which tests must be performed. **Table 10** provides an overview of the experiments that are required, together with an estimate of the duration and the cost associated with this. Finally, in some cases it is still advisable to carry out an additional pilot test on site before the full-scale remediation (3.4.3).

3.4.1 Feasibility analyses

The decision in **Figure 16** can be used as a tool to determine which feasibility analyses are required for specific phytoremediation cases. The laboratory experiments that are required for these feasibility analysis/analyses are described in 3.4.2.

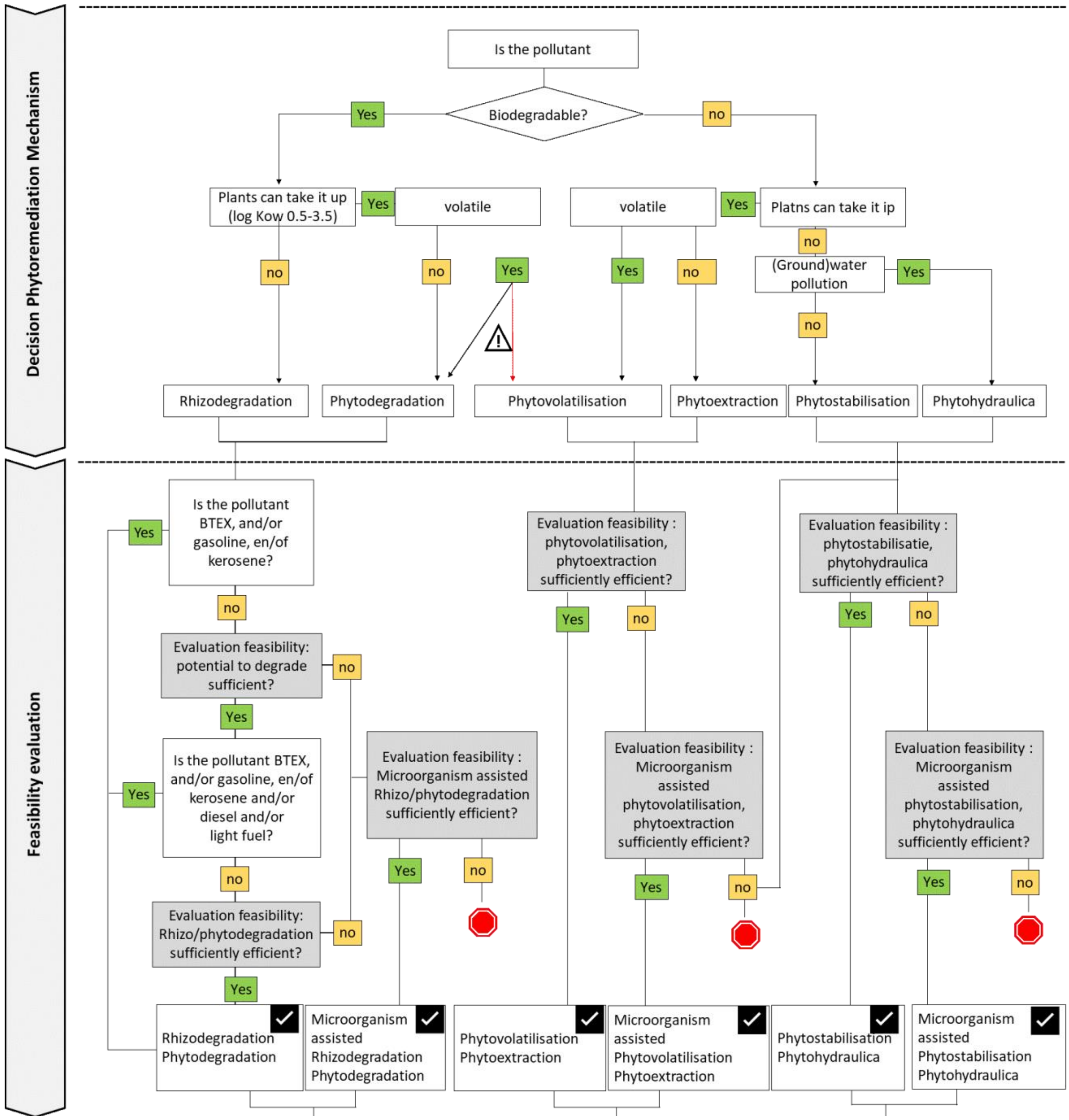


Figure 16: Decision tree for feasibility analyses.

3.4.1.1 <u>Rhizodegradation and Phytodegradation</u>

Based on extensive experience for BTEX, gasoline and kerosene contaminants rhizodegradation and phytodegradation can be applied on site without extra testing. For diesel and light fuel oil contaminants, phytoremediation can be applied after it has been demonstrated that sufficient degradation potential is present. To be able to properly estimate the effective composition of the mineral oil (type of kerosene, gasoline, diesel, etc.) and therefore also the degradability, it is best to use the EPK/VPK analysis or oil characterization performed during the investigation (see Section 2.3). For the other contaminants, a sufficient efficiency of the phytoremediation process must be demonstrated in an extensive feasibility analysis. If the efficiency of a “standard” phytoremediation is insufficient, it is possible to switch to microorganism assisted phytoremediation. The efficiency of this microorganism assisted phytoremediation must also be demonstrated with an extensive feasibility analysis before applying the technology at the site.

Feasibility analysis:
Sufficient degradation potential?
In case of phytodegradation and/or rhizodegradation, the degradation that occurs naturally in some cases may be insufficient, which may lead to a less efficient remediation and/or volatilization of the contamination via the leaves into the air. If fewer than 10^5 degrading microorganisms are present per gram of soil or per ml of groundwater, the degradation of the contaminants will be too slow. This situation can occur in nutrient-poor soils or in soils that contain toxic or inhibiting substances. In the event of insufficient degradation, it is possible to stimulate this degradation by ensuring that the degrading microorganisms are enriched. In addition to samples from the soil in the immediate area around the plant roots, samples can also be used from the plants themselves, in particular root and shoot. Bacteria that live in the plant and have the capacity to degrade the contaminant can then be enriched in the plant and ensure that degradation also occurs during the transport of the contaminants to the leaves. The combination of degradation in the rhizosphere and inside the plant can ensure that there is sufficient time for complete degradation before the contaminants reach the leaves.

Feasibility analysis:
Rhizo/Phytodegradation sufficiently efficient?
In case of phytodegradation and/or rhizodegradation, the contamination is removed by degradation in the rhizosphere and/or within the plant. It is important that the contamination is completely and sufficiently quickly degraded. If this is not the case, the removal of the contamination will occur too slowly in case of rhizodegradation. In case of phytodegradation there is a risk that part of the contamination and/or degradation products will be volatized through the leaves.

Moreover, the risk of phytotoxicity with insufficient or incomplete degradation is greater. Phytotoxicity is associated with reduced plant growth, which in turn can lead to a smaller root system and therefore a smaller radius of influence for the contamination, and a reduced pumping capacity.

Consequently, rhizodegradation and/or phytodegradation is considered sufficiently efficient if the majority of the contamination is removed in a relatively short period of time (greenhouse experiment), and without phytovolatilization of the contamination and/or its degradation products.

Feasibility analysis:
Microorganism-assisted rhizo/phytodegradation sufficiently efficient?
If it appears that there is no naturally occurring degradation potential or that the degradation that occurs with “standard” rhizodegradation/phytodegradation is not efficient enough, the degradation can be stimulated by adding the appropriate microorganisms. This is possible with microorganisms that settle in the rhizosphere and/or inside the plant (endophytes). When the contamination is easily taken up, endophytes have the advantage that the contact time between the contamination and the degrading microorganisms is longer inside the plant. For example, it can take hours to a few days (depending on the size of the plant) before the contamination from the roots reaches the leaves.

The efficiency of microorganism assisted rhizodegradation/phytodegradation is evaluated in the same way as for "standard" rhizodegradation/phytodegradation.

3.4.1.2 Phytovolatilization and Phytoextraction

For phytovolatilization and phytoextraction, a sufficiently high phytoremediation efficiency must be demonstrated for all contaminants before applications can be started on site. If the "standard" mechanisms prove to be insufficiently efficient, it can be investigated whether phytoremediation assisted with microorganisms can increase the efficiency. Adding mobilizing substances (e.g. chelators, surfactants, etc.) to increase the availability of (in)organic contaminants in order to achieve an improved uptake by the plant is not recommended, unless it can be guaranteed that there is no risk on leaching to the groundwater. To estimate this, samples must be taken from the leaching water during the experiment in order to check whether the contaminants do not leach faster than they are taken up by the plant.

Feasibility analysis:
Phytovolatilization or phytoextraction sufficiently efficient?
In case of phytovolatilization and phytoextraction, the contamination is taken up by the plant, transported to the aboveground plant parts and, if volatile (for phytovolatilization), evaporated via the leaves to the atmosphere. For efficient absorption by the plant, the contamination must be available to the plant. This means that sufficient attention must be paid to the risk of leaching to the groundwater. Another major risk with phytovolatilization and phytoextraction is limited plant growth due to phytotoxicity. As a result, the root system will not develop optimally and the uptake of the contamination will be limited.

Phytovolatilization or phytoextraction can be considered sufficiently efficient if the majority of the contamination can be taken up, transported to the aboveground parts and, in the case of phytovolatilization, volatilized via the leaves in a relatively short period of time, and this without the contamination leaching out to the groundwater.

Feasibility analysis:
Microorganism assisted phytovolatilization or phytoextraction sufficiently efficient?
If the efficiency of "standard" phytovolatilization or phytoextraction is insufficient, efforts can be made to stimulate the availability, uptake and translocation of the contamination by adding microorganisms with the appropriate properties. Microorganisms that stimulate plant growth can, for example, decrease phytotoxicity and ensure an extensive root system.

There are also microorganisms that produce mobilizing substances so that the contamination is released. The main advantage is that this mobilization takes place in balance with the activity of the plant: more active plants ensure more root exudates, more active microorganisms, more mobilization of the contamination. Or, in other words, the more active the plant, the more mobilization of the contamination, but also more uptake by the plant. The result is that the contamination that is released can be taken up by the plant almost immediately, thereby avoiding the risk of leaching into the groundwater.

The efficiency of microorganism assisted phytovolatilization or phytoextraction is evaluated in the same way as for the "standard" phytovolatilization or phytoextraction. If microorganisms assisted phytovolatilization or phytoextraction also prove to be insufficiently efficient due to insufficient uptake of the contamination, it is possible to consider applying phytostabilization or phytohydraulics. It is then important to go through the decision tree for the plant choice again in order to choose the optimum plant according to the new objective and mechanism.

3.4.1.3 Phytostabilization and Phytohydraulics

Phytostabilization and phytohydraulics require additional feasibility studies for all contaminants. As the purpose of both mechanisms is to prevent the spreading of the contamination, it may be useful to work with stabilizing substances. For example, lime, phosphates, mineral oxides and organic substances can be tested for their potential to fix the inorganic contamination and thus limit absorption and phytotoxicity. This is certainly recommended if signs of phytotoxicity are observed on the site in the naturally present vegetation. Since this falls outside the focus of this phytoremediation code of good practice and because of the enormous variety of types of soil additives, reference is made to the review by Wiszniewska *et al*. (2016) and Vangronsveld *et al*. (2009) for more information.

If the “standard” phytoremediation mechanisms still prove not to be efficient enough, microorganism assisted phytoremediation can provide further improvement.

Feasibility analysis:
Phytostabilization or phytohydraulics sufficiently efficient?

The objective of phytostabilization or phytohydraulics is to limit the risk of further spreading of the contamination. To limit wind dispersal, it is very important that a good, dense vegetation cover is created, which is only possible if phytotoxicity remains limited. To prevent spreading through the groundwater, it is of major importance in soil contamination that the contamination is sufficiently adsorbed to the soil complex. In case of groundwater contamination a good pumping capacity is crucial. Furthermore, care must be taken to ensure that the contamination does not end up in consumable parts of the plant to prevent it from spreading through the food chain.

Phytostabilization or phytohydraulics is sufficiently efficient if it can be guaranteed that the contamination will not be spread via the wind, groundwater or the food chain.

Feasibility analysis:
Microorganism assisted phytostabilization or phytohydraulics sufficiently efficient?

Adding microorganisms that are able to (i) promote plant growth (in order to guarantee a dense vegetation cover, or to realize increased pumping capacity), (ii) to produce immobilizing substances, so that the contamination is taken up more strongly in the soil complex (to limit both leaching into the groundwater and uptake); can offer a possible solution if the traditional phytostabilization and phytohydraulic mechanisms prove not to be efficient enough.

The efficiency of microorganism assisted phytostabilization and phytohydraulics is evaluated in the same way as for “standard” phytostabilization and phytohydraulics.

3.4.2 Laboratory and greenhouse experiments

3.4.2.1 Presence of degradation potential at the site

From different samples the following microbial parameters can be determined: number of bacteria by plating on selective media, fluorescent staining and counting of the bacteria in a soil extract under the microscope, quantitative PCR and DNA fingerprinting. The presence of indicator species for degradation (e.g. *Halomonas* in the case of chlorinated contaminants, *Pseudomonas*, *Acinetobacter* and *Burkholderia* for oil, *Pseudomonas* and *Enterobacteriaceae* for TNT, *Methylobacterium* for methanol) can be determined. The abundance and transcription of specific degradation genes can be determined with qPCR for mono-oxygenases, dioxygenases, phenol hydroxylase and naphthalene dioxygenase. A complete overview of the microbial degradation potential present can be obtained by a genetic analysis of the complete DNA (‘next generation sequencing’). This can be important for new contaminants.

3.4.2.2 Basic pot experiment under greenhouse conditions

Using a simple pot experiment (**Figure 17**), 3 to 5 interesting plant species (or clones, cultivars) can be compared with each other for different parameters. The selected plants are grown for 6 to 15 weeks under greenhouse conditions. Pots with only soil and without plants are also taken as a control sample. To the extent possible, **soil and/or water from the site** should be used for the entire experiment. Experiments should also be performed with the moisture levels and temperature of the soil at the because these factors also influence the duration of remediation. Furthermore, also addition of **soil additives and/or microorganisms** is possible. In this case, a more extensive pot experiment is needed. For the choice and application of soil additives, reference is made to the review by Wiszniewska *et al*. (2016) and Vangronsveld *et al*. (2009).
To choose the best microorganisms, it is useful to evaluate which microorganisms are already available. For example, there are microorganisms that are known for their growth-promoting effect, or bacterial strains are already available that can degrade certain organic contaminants. Another option is to evaluate the natural **naturally present degrading microorganisms** and **enrich these strains by inoculation**. The bacteria can be added in various ways, depending on the plant species. When working with seeds, the inoculation can take place during the germination of the seeds, while willow/poplar cuttings are best inoculated during root development.

The parameters that can be analysed in this (expanded or not) pot experiment range from phytotoxicity, metal uptake and/or sorption capacity, uptake and/or degradation of the organic contaminant, to volatilization. **Table 10** shows a matrix in which the parameters that can be tested for the chosen phytoremediation mechanism are shown. In addition, an indication on cost and duration of the tests is given.

Figure 17: Pot experiments. Photos: Nele Weyens, Hasselt University.

3.4.2.3 Phytotoxicity

To make an estimate of the phytotoxicity on the plant, the growth and development of the plants are monitored during the experiment. The plants can be assessed for germination, root weight and density, above-ground biomass, growth rate and health. Health can be assessed visually (state of the plant, discoloured leaves) or at the cellular level, for example, by measuring the activities of antioxidant enzymes.

3.4.2.4 Metal uptake and/or sorption capacity

In case of phytoextraction, the plants need to absorb as many metals as possible and translocate them to the harvestable plant parts. In case of phytostabilization, the fixation or sorption of the metals in the soil or in the roots is required.

When plants are harvested at the end of the pot experiment, samples of underground and aboveground plant material as well as soil can be collected. Total and plant-available metal contents are analysed in the soil samples. Metal levels in the tissues can be determined via total acid digestion and ICP-OES analysis.

On the basis of these data, a comparison can be made between the various plant species tested with regard to metal uptake and the plant organs where the highest concentrations of metals are accumulated.

3.4.2.5 Uptake and/or degradation of the organic contaminant

When applying phytodegradation and/or rhizodegradation, the contaminant will be degraded in the rhizosphere and inside the plant. To make an estimate of the degradation rate and which intermediates may be formed, the following analyses can be performed.

At the end of the pot experiment, at harvest, samples are taken from the soil, the soil in the immediate vicinity of the roots, the roots and the shoot. Moreover, for this analysis it is very important to analyse the control sample (soil samples from the pots without plants). The soil itself can also contain degrading microorganisms, causing natural attenuation. The proportion of degradation that is due to phytodegradation and/or rhizodegradation can be estimated and compared.

The samples will be subjected to an extraction process followed by a chemical analysis to determine the concentration of the contaminant.

Based on the results, it is possible to estimate what amount of the organic contaminant can be degraded during the experiment. It should be noted, however, that for volatile organic contaminants it is necessary to take into account possible volatilization via the leaves (section 3.4.1.4) before making this calculation.
Based on the concentrations of organic contaminants that are taken up by the plant, it can be decided which applications are feasible for the harvested biomass.

3.4.2.6 Volatilization

If the volatilization of contaminants or metabolites is the objective or a potential concern, then analyses can be performed during the pot experiment to test this. To determine the amount of contaminant that is evapotranspirated via the leaves, the following measuring system was developed at UHasselt (**Figure 18**).

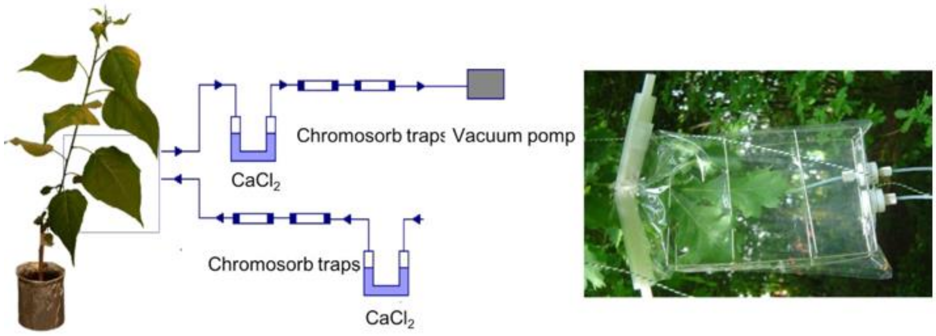


Figure 18: Evapotranspiration measurement system. Photo: Nele Weyens, Hasselt University.

During the measurement, an airtight sealed Teflon bag is placed over a twig so that no gas exchange with the atmosphere is possible. A vacuum pump is connected to 1 of the 2 Teflon gates that are attached to this bag, creating an air inflow. As the air inflow should be completely contaminant-free, the contaminants are adsorbed on Chromosorb traps before the air enters the Teflon bag. If the air leaving the Teflon bag through the other Teflon gate contains organic contaminants, then this is the result from evapotranspiration. The effluent air stream is readsorbed on Chromosorb traps.

The contaminants adsorbed on the Chromosorb traps are quantified via thermal desorption, gas chromatography - mass spectrometry.

The evapotranspired quantities of contaminants are then calculated per hour and per cm^2 of leaf. In this way it is possible to calculate the amount of contaminant that an average plant volatilises from the soil/groundwater to the air per day.

3.4.3 Pilot test

In addition to the relatively short-term experiments, in some cases long term experiments in the field may also be appropriate. This applies when it concerns relatively new contaminants where little or no experience has been gained with phytoremediation.

In long term experiments under field conditions, plants can form sufficient roots and biomass (and possible enzymes). After all, the velocity and efficiency for plant uptake varies with the age (other enzymes, growth rate, etc.) and the metabolic state of the plant (seasonal).

In addition, the addition of microbial inoculants can also be tested under field conditions. In this case plants inoculated prior to planting, can be used. It must be controlled whether the microbial inoculant remains stable over time or whether additional inoculations are necessary. Such additional inoculations can be done via a drainage pipe that is installed in the root system during planting.

Table 10: Matrix that summarizes which laboratory and greenhouse experiments must be carried out for which feasibility analyses, and gives an estimate of the cost and duration.

Phytoremediation mechanism	Feasibility analyses	Lab- and greenhouse experiments						
		Detection degradation potential	Basic pot experiment	Extended pot experiment in greenhouse	Phytotoxicity	Metal uptake and/or sorption	Uptake and degradation of organic pollutants	Volatilisation
Rhizo/Phytodegradation	- Sufficient natural degradation potential?	✓						
	- Rhizo/phytodegradation sufficient?		✓		✓		✓	✓
	- Microorganism stimulated phytoremediation sufficient?			✓	✓		✓	✓
Phytoextraction	- Phytoextraction sufficient?		✓		✓	✓		
	- Microorganism stimulated phytoremediation sufficient?			✓	✓	✓		
Phytovolatilisation	- Phytovolatilisation sufficient?		✓		✓			✓
	- Microorganism stimulated phytoremediation sufficient?			✓	✓			✓
Phytostabilisation	- Phytostabilisation sufficient?		✓	(✓)	✓	✓	✓	✓
	- Microorganism stimulated phytoremediation sufficient?			✓	✓	✓	✓	✓
Phytohydraulica	- Phytohydraulica sufficient?		✓	(✓)	✓	✓	✓	✓
	- Microorganism stimulated phytoremediation sufficient?			✓	✓	✓	✓	✓
	Duration Indicative costs (euro)	10-14d 2k-6k	6-15 w 2k-6k	6-15 w 3k-7k	2-14 d 0,5k-3,5k	5-7 d 1,5k-2,5k	10-14 2,5k-4k d	0 d 2k-4k

* The "(extensive) basic pot experiment under greenhouse conditions" is the basic experiment in which plants are grown under greenhouse conditions that are subsequently used for sampling for further analyses. All these further analyses can be measured on the same basis for a pot experiment, so that the cost/duration of this experiment must only be considered once.

4 PROCEDURE FOR DESIGN, INSTALLATION, MANAGEMENT, FOLLOW-UP AND MONITORING

This chapter contains all aspects for the design and implementation of phytoremediation techniques. It contains information that can help a recognized soil remediation expert to choose a particular phytotechnology based on the conditions on the site and to implement this technology into practice. The most important questions are: what do I have to do to implement phytoremediation and how can I ensure that the system is efficient?

This chapter discusses the all steps to arrive at full-scale implementation of phytoremediation. More specifically:

1) Full-scale phytoremediation system
   a Design and setup of the system
   b Follow-up and process evaluation
   c Monitoring and performance evaluation
   d General legislative framework
2) Remediation objectives
   a Qualitative and quantitative measurements
   b Criteria for success: process & performance criteria

All criteria are of decisive importance to be able to guarantee the necessary quality on the one hand with the customer (remediation obligation) and on the other hand with the supervisory authority (OVAM).

4.1 DESIGN

In Chapter 3 it was already evaluated whether phytoremediation is feasible as a possible remediation alternative (shown schematically in **Figure 12**). This process also forms the basis for the design of the phytoremediation process.

The final design of the phytoremediation project strongly depends on the conditions of the site such as the soil type, concentrations of the contaminant, extent and depth of the contamination. A thorough understanding of these variables is necessary to select the plant species, phytoremediation mechanism, plant schedule, maintenance and monitoring as also described in Chapter 3.

Specific aspects such as bioavailability, phytotoxicity, hydrological control and concentrations of contaminants are important in the design and implementation of remediation systems.

In the following sections, the design specific criteria are discussed or summarized briefly if they were already covered in Chapter 3.

4.1.1 Phytoremediation specific field characterization

The design requires a thorough field study.

A description must be given of the main phytoremediation specific field parameters as included in the feasibility screening in section **3.1.1 Phytospecific site characterization**. It should be indicated how these parameters will be included in the design.

4.1.2 Contaminated medium

The design includes a description of the medium (soil/ sediment/ groundwater) and the associated criteria:
- the depth and volume of the contaminants
- the soil characteristics such as texture, water content, nutrient content, pH and permeability, which determine whether or not the contamination is accessible to the plant.
  - Many contaminated sites consist of soils that have a **low nutrient content** and are often highly compacted. In these cases, prior soil treatment may be necessary to achieve successful planting. The need should be described in the design.
  - The presence of **less permeable/less penetrable layers**, for example a clay layer in a sandy soil or sandstone layer, can influence the development of the roots. These layers can be interrupted using tree wells or tree sleeves as shown in **Figure 11**. The need should be evaluated in the design and further elaborated if relevant.

4.1.3 Contamination

The preconditions were explained in section 3.1.1.2. These preconditions must be further elaborated for the site specific design.

The design depends on the concentrations and type of contaminants:
- In which zones phytoremediation is feasible?
- In which zones are additional active measures required?

4.1.4 Vegetation present

In certain cases, **vegetation** is present on the contaminated site or on an adjacent site (**3.1.1.3. Vegetation present**).
The design must indicate whether the following aspects have been derived from the vegetation present on site and how this is taken into account in the design:
- the root development, evaluated in a trench on site: if limited root development is present, the specific selection of the plant (see 4.1.4) and planting distance is of major importance. Root depth and density can also be determined on site.
- the vegetation present at the site: the design must indicate whether the present vegetation which is tolerant for the contaminant, can be used for the remediation. On the basis of a literature study it can be determined which species are tolerant to the contamination present and can have the desired effect for the degradation, extraction or stabilization of the contamination.

4.1.5 External factors

The design must include a description of the most important external factors and indicate how this is taken into account in the design:
- What space is available and what is the current and future use of the location and adjacent plots?
  - Description of contaminated zone and spreading velocity of the contamination in groundwater and space required for this

- Combining multiple user functions can provide specific added value for a phytoremediation project. That is why it is important to have insight into the current and future use of the site and the use of the surrounding sites.
- If there are nature management plans for the surrounding sites present, a specific layout of the contaminated site can contribute, either by conducting a similar management or by choosing another layout that increases the ecological value for both sites.
- Involving owners, users and nature managers of surrounding sites can increase the social support for phytoremediation.

– How will underground/aboveground obstructions taken into account in the design?

4.1.6 Remediation objectives

The remediation objectives of a remediation project can consist of a containment or a mass removal of the contaminants, or a combination of both.

If the primary objective is the decrease of spreading risks (f.e by using phytostabilization or phytohydraulics), the remediation objective can be based on limiting soil filtration due to rainfall (in the case of phytostabilization) or optimizing the uptake of groundwater as well as evapotranspiration in case of phytohydraulics) by a correct selection of the plant.

Containment of a contaminated zone can consist of a soil cover by planting as well as an hydraulic barrier (usually downstream of the contaminant plume, or upstream to reduce the groundwater flow rate and thus the velocity of the contamination). In the case of containment, the remediation objective is a stable contaminant plume.

Phytotechnologies based on removal of the contamination are f.e phytoextraction and phytovolatilization. The degradation of contaminants can be achieved by rhizodegradation and phytodegradation. The applications for the removal and/or degradation of the contaminants are, for example, phytodegradation and rhizodegradation soil coverings, plants that can remediate deeper soil and groundwater, reed beds and related systems.

The applications of phytotechnologies that combine both containment and removal of the contamination can consist of coverings that stabilize the soil and phytoremediate the contamination, a planting as a hydraulic barrier that at the same time remediates the soil and groundwater in this zone, reed beds and related systems including bank plantations that can prevent the contamination from spreading to surface water. An example of this are banks with willows, Reed (*Phragmites australis*), Cattail (*Typha*), Yellow Iris (*Iris pseudacorus*), etc.

4.1.7 Phytoremediation mechanism identification

To help with this decision process to select the correct phytotechnology, a decision tree has been created (**Figure 16**). The use of this decision tree, along with the this whole document, can help supervisors, site owners, soil remediation experts, soil remediation contractors and the public in determing whether phytotechnologies can be applied to a contaminated site. Nevertheless, the decision tree will mainly be used to make technical decisions.

However, the selected remediation technique also depends on other factors such as:

– remediation objectives;
– expected effects;

- acceptance by stakeholders, site owners, regulator and public opinion;
- costs.

In addition, combining phytotechnology with another (existing) remediation technique on site can also be useful. In that case, the effects of both systems should considered.

4.1.8 Plant selection

In Chapter 3 (**3.3 Identification of most suitable plant(s)**) the importance of the correct choice of planting was extensively discussed. It is important here to use the decision tree (**Figure 15**).

During the feasibility study, the outcome of this decision tree yielded a list of species that may be applicable for phytoremediation. Possibly this was refined on the basis of a thorough feasibility analysis (**3.4 Thorough feasibility assessment**) such as laboratory, greenhouse and field experiments. Based on all available information and/or test results a final plant selection for the site should be made.

The final choice of the plants, which will be included in the design, will be based on a number of important parameters such as type of contamination, risk of contamination spreading, location, etc., but also on the specific requirements of customers.

4.1.8.1 Contamination

The vegetation present at the site can provide useful indications of possible species that are tolerant to the contamination. Furthermore, based on a literature study, species that are tolerant to the contamination present and can have the desired effect on the degradation, extraction or stabilization of the contamination present can be selected.

In addition to the type of the contaminants, the depth of the contamination is also important. For example, in case of deep contamination, the use of deep rooting plants will be necessary to have sufficient remediation efficiency. It is of course always possible to combine phytoremediation with other remediation techniques to have a sufficient efficiency on the deeper contamination. For example, groundwater can be pumped up and then infiltrated into the root zone.

4.1.8.2 Risk of spreading the contamination

When choosing a plant, account must be taken of any risks of spreading the contamination via the food chain. For example, it may be advisable not to use plants that produce edible parts for humans or animals when the contamination ends up in these parts of plants. This is, for example, the case when applying phytoremediation to areas that are publicly accessible. In the case of phytoextraction, leaf fall can pose a risk of spreading.

It is necessary to identify the relevant risks of spreading and, where appropriate, to coordinate the monitoring accordingly.

4.1.8.3 Location

The location has certain characteristics such as the soil type (sand, loam, clay, etc.), the pH, the nutrient content, the moisture content, the light, etc. Plants that are not suitable for a certain location may not grow well, are susceptible to diseases and suffer sooner from the phytotoxicity as a result of the soil and/or groundwater contamination present.

4.1.8.4 Attention to layering
When applying the forest succession stage, the aim is to preferably achieve a layered structure consisting of a tree layer, shrub layer, herb layer and litter layer.

4.1.8.5 Habitus
In the remediation design, the habitus of the plant should be taken into account. This habitus is determined by the dimensions and the growth form as well as the properties of the roots. The depth of the roots depends, among other things, on the species and will help determine whether or not the contamination can be reached by the plant. Trees with superficial roots can also push up pavements and root shoots can grow through the joints of pavements.

4.1.8.6 Origin
Wild plants are plant species that occur in natural or semi-natural vegetation and can survive without human intervention. Cultivation varieties were created through the cultivation and selection of wild species. Cultivars are not always able to cope with competition from wild species, which can result in intensive nature management.

Native species are preferred because they are well adapted to local soil and climate conditions and their life cycle is closely linked to that of many native organisms. Choosing native species increases the ecological value of the planting.
It is clear that non-native species can be chosen based on specific phytoremediation characteristics. In that case it is important that non-invasive species are selected or that the necessary measures are taken to prevent the spread of these non-native invasive species into the environment. Information about **native plant species** and other characteristics can be found in the vademecum of “Agentschap Natuur en Bos” (Flanders).

A major advantage of cultivated varieties is that the seeds and planting shoots are often readily available and are cheaper than the native species. Furthermore, due to years of selection, these cultural varieties are sometimes less susceptible to diseases, climate conditions and other factors that can limit growth. Furthermore, fast-growing or more biomass-producing varieties are often available that can be selected with other specific characteristics. Thanks to these advantages, many poplar and willow hybrids have already been extensively and successfully used in phytotechnology processes.

In addition, we should opt for **pesticide free management** of greenery and pavements. Where possible, we also strive for **ecological added value** and combining multiple functions for the site.

4.1.8.7 Other properties
There are many other properties that can determine the final selection of the plant. The sensitivity to branch breakage, for example, must be taken into account when planting at a parking lot. The sensitivity to diseases is also important in function of a successful planting.

4.1.9 Alignment of the design with the specific needs of the feasibility study
Planting trees is unfortunately no guarantee for effective soil and/or groundwater remediation. Control and follow-up by humans are necessary. The success of phytoremediation depends on the type of plant, but also on the microorganisms present and possibly the use of additives.

If the feasibility study, which may or may not be extensive, shows that optimization of the phytoremediation process is necessary, for example because of a risk of volatilization or if monitoring shows that significant volatilization occurs, the phytoremediation process should be improved, for example by adding the desired microorganisms. Optimization may also be needed to increase the efficiency of the phytoremediation process to, for example, shorten the duration of the remediation or reduce any phytotoxicity. When designing, it is important to meet the specific needs for optimization. For example, it is necessary to install a drainage system when working with microorganism assisted phytoremediation.

Soil additives
Different types of additives can be used depending on the purpose. If the aim is to stabilize the contamination, additives such as lime, phosphates, mineral oxides and organic substances can be used. However, if an increased uptake of the contaminant (organic or metal) is desired, mobilizing substances (e.g. chelators, surfactants) can be added that increase the availability of the contaminant. However, this is strongly discouraged unless it can be guaranteed that no leaching will occur into the groundwater.

Microorganisms
If the degradation of the organic contaminants is insufficient, this can lead to phytotoxicity if the concentrations are too high, causing the plant to exhibit inadequate growth or even to die. Moreover, if it concerns a volatile contamination, there is the additional risk that the organic contaminants will evapotranspire through the leaves to the air (phytovolatilization). Both problems can be solved by providing the appropriate plant associated bacteria. Endophytic bacteria, which naturally live inside the plant, if 'equipped' with the appropriate degradation mechanisms, help plants to survive in situations with increased levels of contaminants are present and increase the capacity of the plants used to degrade these contaminants and consequently remove them from the environment.

An enrichment of these bacteria in the plant therefore optimizes the phytoremediation process. This can be achieved by improving the growth and health status of the plant, and also by microbial transformation of contaminants into products less harmful for the plant. In addition to promoting degradation of organic contaminants, microorganisms can also be added to promote the uptake of contamination (metals and organic contaminants) (e.g. bacteria that lower the pH in the root zone or produce surfactants that mobilize oil) or to limit it (e.g. bacteria with metal-immobilization mechanisms).

The necessity to add microorganisms results often from the feasibility study, but can also be seen from the results of the monitoring.

4.2 SETUP

The site will always require some pre-treatment before the ‘start’ of the phytoremediation.
For plant covers, this will often be the entire planting area/contaminated zone. In case of trees pre treatment is often limited to a tree zone or a trench. This soil pre-treatment for phytotechnologies is often similar to that for agriculture and horticulture. The setup of the site will in many consists of preparing the soil for planting, fertilizing, sowing or planting the desired species and irrigating.

The specific requirements for these soil pre-treatments vary from site to site and should be aligned with, among other things, the soil characteristics that were determined during previous surveys and site visits.

In many former industrial areas, the soil will often be compacted. This is of course disadvantageous when applying phytotechnologies because these compacted soils prevent the roots from penetrating sufficiently. In this case, this soil must also be ploughed and harrowed beforehand. The depth here depends on the degree of compaction of the soil. In most cases, it is sufficient to loosen the top 50 cm of the soil to achieve good root development. If planting holes and/or trenches must be installed for planting, this full depth of soil must be routed. An additional advantage of this soil pre-treatment is that oxygen is introduced into the soil. Especially with organic contaminants this is recommended for stimulating aerobic biodegradation.

Both during and after ploughing, any necessary fertilizers can be incorporated. Furthermore, depending on the timing of planting, it may be necessary to provide irrigation. This is mainly the case with trees that are planted in dry weather in the spring.

In the following pargraphs, six different **plant typologies**, or design strategies, are described, as well as the control measures proposed by the Agency for Nature and Forests (Agentschap Natuur en Bos, Flanders) that can be used for the implementation of the processes. The different phytotechnology plantations can be used either individually or in combination. Some plant typologies are only suitable for certain contaminated substances or for certain target media (soil, groundwater, surface water).

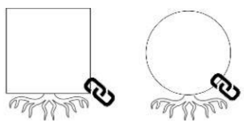
A system of **pictograms** is used at the start of each typology to quickly identify which contaminant and target media are treated with a particular planting type and which **phytoremediation mechanisms** are used. Furthermore, a diagram and description of each planting type have been added with notes about typical applications and plant selections.

When going through the plant typologies, the plants themselves should be selected on the basis of the described criteria and the specific site conditions, including the soil, groundwater, microclimate and contaminants present. The typologies must be used with the plant lists as can be found in **Appendix 4** and the **phytoremediation database**. Although the plant typologies are discussed separately below, in reality different typologies can be combined in a planting scheme to achieve multiple remediation functions and objectives.

4.2.1 Stabilizing plant cover

Description: The plants retain the contaminants and prevent migration and leaching. There is no direct removal of the contamination. The aim of stabilizing plan cover is primarily to reduce exposure risks to humans and the environment.

Mechanism: phytostabilization



Target medium: soil including groundwater

Target contaminants: This plant typology is most often used for contaminations with heavy metals, POPs and salts, but can be used to a certain extent for all contaminants.

A stabilizing plant cover is similar as a clay cover, it fixes the contamination at the site and minimizes the exposure to people and the environment. The difference is that plants prevent contamination from leaching, but they still allow water to penetrate into the soil. Plant roots retain the contamination and root exudates can additionally bind the contaminants to soil particles and organic matter.

A stabilization plant cover is most often applied to contaminated sites with non-bioavailable contaminations and where vegetation is a priority. Plant species are selected that are highly tolerant to the contamination and soil additives can be added to improve plant growth and to capture contaminants. In phytostabilization, "excluder" plants are used that do not transport the contaminants to the aboveground plant parts, but that prevent wind and soil erosion. Moreover, the pumping of water through the plants reduces the risk of the leaching of contamination into the groundwater. Finally, stabilization plant cover create habitats for animals and insects.

Typical application

Lead contaminants: A large amount of lead may still be present in soils around houses that were painted with lead paint, which was permitted up to the 1970s. In addition to the earlier use of lead-containing paints, firing ranges are also often contaminated with lead. Lead is very difficult to mobilize in water and can remain in the soil forever. The most common route of exposure to humans is through dust particles that we breathe and that we bring home via our shoes. If the contamination is extensive, excavation is often not feasible. In this case phytostabilization with a plant cover offers a solution. The plant cover forms an effective barrier between soil particles and the inhabitants.

Plant selection
For stabilization plant covers, plants that are tolerant to the contaminant, that can fix the contaminant and that can form a dense vegetation cover should be used. The plant decision tree in **Figure 15** must be followed to select at the plant, namely (1) are plants already growing at the site and are these known in the database to be suitable for this site, (2) is there a species already in the database suitable for the terrain, (3) are certain clones, varieties known that are the most efficient, (4) can the plant survive the contamination and terrain conditions.

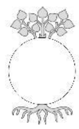
Figure 19: Example of phytostabilization Lommel-Maatheide (Zn, Cd, Pb) contamination. A: Condition before phytostabilization; B: Removing debris; C: Fertilization; D: 2 weeks after sowing; E: 5 years after sowing; F: 12 years after sowing. Photos Jaco Vangronsveld, UHasselt

Setup tips and suggestions

1. *How can a grass stabilization cover with zero exposure be installed*? Choose plants that demonstrate very dense growth and that leave little or no soil exposed. Grass species are often best suited for this application because of their capacity to form dense sods. Thick, densely sown *Festuca* and *Agrostis* species are often a good choice for this application, especially for park areas and residential environments with lead in the soil.
2. *Sowing grasses*: We recommend that suitable grass types are sown. We do not recommend spontaneous grass because it may take a long time to become a dense vegetation. The stabilizing effects would therefore only occur with a delay. It may be advisable not to sow flowering grassland plants in the most polluted areas to avoid the risk of spreading through food cycles. In the less polluted zones, grassland plants may (also) be sown.
3. *Soil chemistry*: With stabilization covers, soil chemistry is at least as important as the choice of the plants. Precipitation, sedimentation and binding to the soil particles can also play a major role in remediation. It is therefore important in some cases to adapt the soil chemistry, e.g. the pH, the availability of nutrients. In the case of excessively toxic concentrations of contamination, the contamination can also be fixed by additives. An agricultural and horticultural expert can be consulted for the choice of the best soil additives.
4. *Fertilizers*: Organic fertilizer can be added to the soil to bind the contaminants and to promote plant growth.
5. *Contamination type and concentration*: Not all contaminants can be stabilized equally efficiently with a vegetation cover (**Figure 19**). Sometimes the contamination concentrations are so high that no plants can grow. In other cases, the contamination may be soluble in water. Since vegetation covers do allow water to pass through, the contamination can still be mobilized despite the cover. Monitoring of groundwater and run-off water at regular intervals is recommended. In some cases, legislative requirements may also require other remediation options.

4.2.2 Degrading plant cover

Description: Hedges, shrubs, shrubs, deep rooting grasses and herbaceous plants can be planted to absorb and degrade contaminants in the soil to a depth of one metre. The contamination is removed by degradation without the plant having to be harvested.

Mechanism at work: rhizodegradation  and phytodegradation 

Target medium: surface soil and water (0 - 1 metre)

Target contaminants: organic contaminants: mineral oil, chlorinated solvents, pesticides and herbicides, nutrients such as nitrogen, POPs and explosives at military sites. Also useful for the removal of free cyanides.

Hedges and hedgerows can be used to fence off contaminated locations at a site. The plants and their associated microorganisms degrade the contaminants into less complex, less harmful substances in the root zone, stem or leaves. The choice of plant species can influence the rate of degradation. Some plant root exudates have similar chemical structures to the contaminants themselves.

Bacteria that live in the root zone of these plants therefore often have more potential to use the structurally similar contaminants as food instead of the root exudates. Each plant species also secretes a species-specific range of root exudates that will affect the associated microbial communities. Hedges, shrubs, grasses and herbaceous plants can be very successful in degrading certain mineral oils, chlorinated solvents and pesticides in the soil.

They can sometimes also absorb high concentrations of nitrogen from the soil. Some forms of nitrogen (nitrate, ammonium) can be metabolized by the plant and converted into biomass.

Typical applications

Contamination around underground fuel oil tanks in gardens:
If an underground tank has leaked, hedges and shrubs can degrade -after source removal- the remaining contamination in the soil (see section 2.3).

Manure accumulation/disposal: At locations where there is too much manure in the soil, plants can be used to absorb the nutrients and incorporate them into biomass.

Areas around petrol stations, garages, industrial sites in the city, as separation between road and cycle path:
Hedges can be planted around properties and roads to degrade organic contaminants in the soil (and also air). The plant buffer can also have aesthetic advantages to reduce unwanted views and to determine property separation.
Mixed plantations can certainly also be considered here to perform multiple ecological functions such as habitat and green connecting zones between nature reserves.

Hedges around gardens and fields: Hedges can be planted around community gardens and fields to degrade organic contaminants in the soil and prevent large amounts of nutrients and pesticide runoff. In addition, hedges can also offer aesthetic benefits and protect agricultural and horticultural crops against vermin and pathogens.

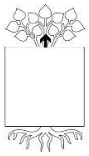
Plant selection: Plants that are tolerant to the contaminant must be able to degrade the contaminant and be able to root (moderately) deeply. The plant decision tree can be used for this selection. Consult the database to see if similar contaminants are already known and which plant species were successful. It is important to evaluate whether the plants do root deep enough to reach the contamination. The plants are also preferably easy to grow from cuttings and can easily be pruned into shape for maintenance. Willow species may be very suitable for this, depending on the contaminant.

Setup tips and suggestions
1. *Layering*: To achieve maximum degradation of the organic contaminant and nutrients, hedges can be alternated with other herbaceous plants, grasses and other plant typologies around the edges of the contaminated site.
2. *Maintenance*: It is usually sufficient if hedges are pruned back into shape once a year.
3. *Irrigation*: in hot summer months it may be necessary to irrigate phreatophytes such as willows, especially at planting and until they are well rooted in the soil at the site. Once properly rooted, most species are drought tolerant. The water regime can be taken into consideration when choosing suitable plants.

4.2.3 Extractive plant cover

Description: Hyperaccumulators and plants that can generate high biomass are often used to remove inorganic and difficult organic contaminants from the soil. The plants must be harvested to remove the contamination from the site.

Mechanism at work: phytoextraction



Target medium: surface soil (0 - 1 m)

Target contaminants: Depending on the initial concentration and bioavailability in medium term (at least 10 years) elements such as arsenic (As), selenium (Se) and nickel (Ni); in the longer term metals such as cadmium (Cd) and zinc (Zn).

Not currently suitable for: cyanide, radionuclides, salts and the following metals: B, Co, Cu, Fe, Mn, Mo, Cr, F, Pb, Hg, Al, Ag and Au.

Not applicable for: mineral oil, chlorinated solvents, pesticides and explosives. All these organic contaminants can be remediated by means of degradation mechanisms. Phytoextraction is therefore not recommended here.

The harvested material must be analysed on contaminant concnetrations before it is properly disposed of (in accordance with VLAREMA in Flanders). The inorganic contaminants (other than Arsenic, Selenium and Nickel) are usually taken up in insufficiently high concentrations to achieve the remediation objective in an acceptable time frame.
However, if the time frame is not a limiting factor, it may still be interesting to perform phytoextraction. For a site moderately contaminated with Cd and Zn, for example, phytoextraction was already applied using poplars and willow clones. In the short term phytostabilization is the main mechanism and phytoextraction only only takes place in the long term.

Typical applications

Selenium: Selenium always occurs in the soil. In some areas, however, higher concentrations occur that can leach into the groundwater and therefore can have a negative impact on human and animal health. Increased concentrations can also occur as a result of mining and intensive land use. Various plants are known that can absorb Selenium, some can even volatilize it. In certain cases, it is not necessary to harvest the plants because most of the Selenium has volatized. If the plants are nevertheless harvested, they do not always have to be treated as waste. Since Selenium is an essential micronutrient for animals, the plants could therefore be given a useful purpose.

Phytomining of Nickel: Nickel is one of the few metals where hyperaccumulators are effectively used to extract the metal from the soil. Since there is a high demand for Nickel, there is therefore the potential for removing Nickel from the soil using plants.

Large industrial areas of metal smelters: Where the contamination is not yet too deep in the groundwater, hedges and shrubs can absorb the contamination.

Long-term remediation of agricultural and horticultural areas: Agricultural and horticultural areas may already be contaminated with metals due to surrounding mining activities, intensive industries and naturally occurring high concentrations in the soil. Here, the bioavailable fraction in particular poses a potential risk to food crops because of the increased concentrations in the consumable parts of the plants and because of the growth inhibition of the plants. In addition, animals that eat these contaminated crops can bioaccumulate the metals, leading to even higher metal concentrations in the food chain. A potential long-term application can then consist of reducing or removing the bioavailable part of the metals from the soil using phytotechnologies. However, the soil still remains contaminated, but the bioavailable fraction of the contamination that previously entered the food chain can be reduced or removed. This extractive plant cover can be used for many years. As soon as the bioavailable fraction has been removed, it is possible to switch to the cultivation of food crops. The equilibrium between metals that are bound and that are available is a dynamic system: if a large part of the bioavailable fraction is removed, the metals that were initially bound to the soil will become available.
These shifts in equilibrium continue until only those fractions of metals remain that are so tightly bound that they will no longer be available. A stable equilibrium must therefore be maintained before the phytoremediation process can be stopped.

Another use of extractive plant covers in agricultural and horticultural areas can consist of completely replacing the food crops with hyperaccumulators that slowly (usually over a period of many decades) remediate the soil. A periodic harvest is essential for this. Biomass crops such as grasses, willows and poplars have already been evaluated as energy crops. Due to the continuous harvesting of the aboveground parts, the site is gradually being remediated.

Plant selection
Hyperaccumulating plants are selected for contaminants with Arsenic, Selenium and Nickel. An important observation is that, although many plants are capable of absorbing metals, they cannot reduce soil concentrations sufficiently. The metals have limited bioavailability and/or are too strongly bound to the soil matrix to be extracted. For inorganic contaminants, phytotechnology that keeps the contamination at the location, but eliminates the risk of human exposure, is therefore often the best option. Together with a stabilizing plant cover and a hydraulic barrier, contaminants can be successfully kept at the location.


Figure 20: Extraction of plant cover with willow and poplar and short rotation woody crop harvesting. Photos: Jolien Janssen, Hasselt University.

Setup tips and suggestions

1. *Bioavailability*: Many inorganic contaminants such as metals and radionuclides occur in the soil in a form that is hardly available for plants. This means that the extraction of these substances takes a long

time, but it also has the advantage that the concentrations of these substances in the biomass can remain sufficiently low so that various more common applications of this biomass remain possible.
2. *Biomass removal*: Once the inorganic contamination has been taken up by the plants, they must be harvested every year to remove the contamination. The correct destination must be selected depending on the concentration in the plants. This is explained in Section 4.3.5. Processing of the biomass.
3. *Risks and bioaccumulation*: In contrast to degradation typologies that completely remove the contamination, an extraction plant cover will move the contamination to a large extent from the soil to the aboveground plant parts. Contaminants then become available for consumption by insects, animals and other predators. This mobilization of the contamination can therefore create new forms of exposure. In this case, additional experiments must be performed to determine whether exposure to the contamination can be prevented and whether bioaccumulation poses a risk. Potential risks can be prevented by a specific plant choice where the relevant plant parts are not part of the food chain. This is often the case for noon-native species. If this is not possible, a fence can offer a solution.

4.2.4 Hydraulic barrier with phytodegradation and phytovolatilization

Description: Trees that have deep roots and can evaporate a lot of water are planted to influence the groundwater flow and to limit the migration of contaminants. The rapidly transpiring trees can delay or even stop a groundwater plume. They can even divert the flow of the groundwater plume towards the trees. The aim is often therefore to prevent contaminated groundwater plumes from leaving the site. An additional advantage is that many organic contaminants can be degraded and/or removed with this process.

Mechanism at work: phytodegradation , phytovolatilization, rhizodegradation , phytohydraulics

Target medium: soil and groundwater

Target contaminants: organic contaminants (mineral oil, chlorinated solvents, nitrogen) because they can be degraded; explosives, POPs and metals can be contained (no degradation).

Using trees for a hydraulic barrier makes use of “pumps” that are powered by solar energy.

Typical applications

Industrial sites contaminated with chlorinated solvents and volatile organic components: Chlorinated solvents such as TCE and PCE often have a high flow rate in the groundwater. They can therefore spread quickly and are often difficult to completely remove with conventional groundwater remediation techniques. Phytohydraulics by means of a hydraulic barrier with trees is an ideal way to clean up widespread contamination plumes. The trees not only control the flow of the contamination plume but, due to the biological activity in the trees and in the root zone, they will also degraded the contamination.

Dry cleaning: TCE and PCE are typical contaminants from dry cleaning, together with other chlorinated solvents. They are mobile in groundwater and can easily be pumped up and removed (degraded) by means of a hydraulic barrier.

Fuel tanks, petrol stations and oil refineries: Light fractions present in fuels such as BTEX and MTBE can migrate quickly into groundwater. Leaking storage tanks are a common environmental problem. A hydraulic barrier can therefore stop and degrade the migration of this contamination.

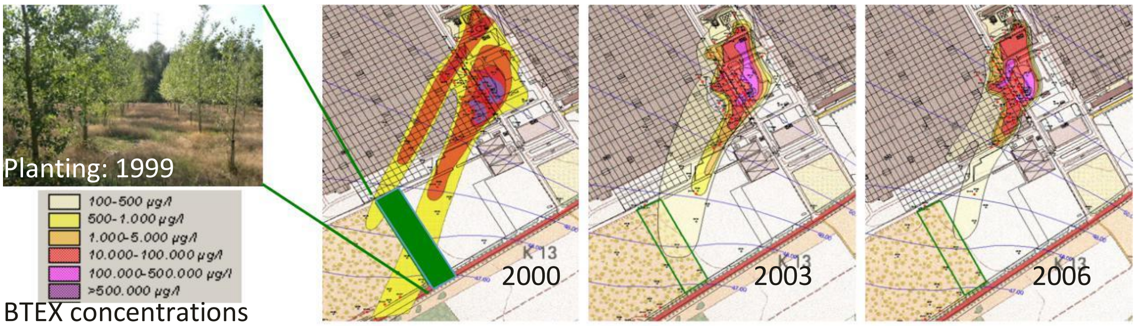


Figure 21: Phytoremediation of a BTEX contamination plume at Ford Genk (Practical example 7). Photo Nele Weyens: Hasselt University.

In Genk, a BTEX contamination was remediated by a combination of groundwater remediation (pump and treat) and soil vapour extraction in the source zone and phytoremediation in the plume zone. The planting of a poplar field took place in 1999. After 4 years the plume was reduced and after 7 years the concentrations of BTEX under the poplar field were below the detection limit (**Figure 21**).

Plant selection

Plants that are tolerant to the contaminant, that deeply rooted and also absorb a lot of water are selected. Phreatophytes are advised as, depending on the depth of the groundwater table, they can easily root up to 9 metres deep.

To ensure that the system works smoothly, the hydraulic barrier is usually used when the groundwater is at a maximum depth of 6 metres. Less deep groundwater is reached easier and faster by the trees. Since it can take a certain amount of time before the roots of the trees reach the groundwater, it is sometimes advisable to plant the trees deeper. This can be done by means of a mechanical auger. In this way trees can be planted up to 3 metres deep. However, this is not necessary in most cases.

Setup tips and suggestions

1. *Management*: The distance between the hydraulic barriers (screens of trees perpendicular on groundwater flow) must be sufficient to prevent spreading of the contamination when the plants are “less active” (winter). A distance that is preferably at least twice the distance covered by the most mobile component of the contamination per year is advised. We also take into account any space required for carrying out management work and possible damage that the planting can cause to infrastructure such as buildings, pavements, underground pipes, etc.
2. *Planting trees*: It is best to plant trees between November and mid February. It is recommended to mix plants in groups when applying different types. Depending on the situation, we opt for forest planting material, larger plant sizes, whether or not container plants, cuttings/pots. Sometimes it is necessary to carry out the planting work in such a way that the plants start to root to a desired depth. This can be done by planting in boreholes or by using so-called tree sleeves. The planting densities are species-dependent and are also determined by other factors such as, for example, the minimum width of passageways for maintenance. It is advisable to plant under the trees also shrubs or herbaceous plants that are suitable for the growing conditions.

4.2.5 Multimechanism design

Description: A mixed planting that uses many, or even all, phytotechnology mechanisms. The aim is to achieve maximum phytoremediation benefits over a large area with a mixed contamination of organic and inorganic components.

Mechanism at work: phytoextraction , phytodegradation , phytostabilization , phytovolatilization

Target medium: soil (0-1 metres deep) and groundwater

Target contaminants: all

A multi-mechanism plant cover is installed with all phytotechnology mechanisms in mind, phytoextraction, hydraulics, degradation, volatilization, stabilization through a low shrub, rugged planting that maximizes phytotechnology impact and minimizes the risk of exposure.
Elements of stabilization plant covers, extraction plantations, hydraulic tree rows, and degradation hedges are combined to create a multifunctional, dense planting at the site. Phytoextraction plots should be harvested at the end of each growing season to remove a maximum of contaminations from the site.

Typical applications

Train verges, abandoned industrial sites: Seed mixes can be composed with extraction and/or degradation types.

Military base, firing ranges: Contaminants including explosives, mineral oil (such as kerosene), chlorinated solvents, metals (lead, copper) are historical contaminants, still present today because of their persistent nature. In addition, new contaminants are also added through training activities. Areas that are no longer actively in use due to lost soil structure (e.g., former DOVO zones) can be restored by low vegetation plantations, roughness, shrubs and grasses that do not interfere with firing practice (Figure 20). Higher rows of trees can be provided on the edge as a buffer and to limit viewing. Extraction and degradation plant species can be considered to increase the functionality of the phytoremediation mechanisms.

Plant selection
Plants that are tolerant of the contaminants, that occur in databases to effectively remove or fix the specific contamination.

Figure 22: Multi-mechanism on military firing range in Helchteren. Left, DOVO dead zone. On the right, grassland at a recovering location and rows of trees that form a buffer in the distance. Photos: Sofie Thijs, Hasselt University.

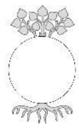
Setup tips and suggestions

1. *Ecosystem services*: In addition to remediation, these multi-mechanism plant covers also offer protection against erosion, stimulate the occurrence of wildlife, the aesthetic character and CO_2 capture in the soil. The mixed plant composition in these buffers creates opportunities for species diversity and multi-level ecosystem services (see also sustainability).
2. *Biomass production*: the biomass that is harvested in these areas can generate income, such as production of wood or bioenergy (see Section 4.3.5 Processing of the biomass).

4.2.6 Biotreatment systems

Description: In a horizontally constructed wetland or a biotreatment plant, water contaminated with organic or inorganic substances can be drained and purified in the root zone of the plants. A choice can be made from different substrates to remove the contaminants.

Organic contaminants and nitrogen can be completely removed and degraded and other inorganic contaminants can be filtered from the water or fixed in the soil.

Mechanism at work: rhizodegradation  and phytodegradation 

Target medium: waste water, rainwater, groundwater, rinsing water from spraying installations in agriculture and horticulture

Target contaminants:
Substances that are broken down/removed: nitrogen, certain mineral oils (see Section 2.3), chlorinated solvents, pesticides

Substances that are captured in plants: explosives, most metals, phosphorus, POPs

Substances extracted into harvestable parts of plants: some metals, phosphorus, nitrogen.

Reed beds, coastal wetlands and other "constructed wetlands" mimic natural ecosystems and use plants that filter contaminated water through the root zone. Most treatment processes for the contaminants do not occur in the plant, but in the biofilm around the roots. Plants act as a carrier for microorganisms and provide the system with organic material, oxygen, nutrients, sugars and other root exudates.

The design of bio-treatment plants is very specific and has already been discussed in detail in other publications (https://www.pcgroenteteelt.be/en-us/Actueel-nieuws/zuivering-van-restvloeistoffen-van-het-spuittoestel-praktische-leidraad-1 in Dutch).

The water management and media must be chosen by specialists in order to achieve the desired degradation rates. If a wetland functions poorly, it can in many cases be attributed to the design, implementation or maintenance.

The most important treatment processes that take place in a biotreatment plant are:
- Many organic contaminants can be degraded

- Nitrogen is removed as gas via denitrification by anaerobic microorganisms
- Inorganic contaminants are filtered from the water or stabilized in the soil instead of in the plants. The contaminants remain in the soil and the water is treated.
- Phytoextraction is usually not the goal because the plants then have to be harvested to achieve removal of metals.
- Nitrogen and phosphorus are converted into plant biomass. The nitrogen and phosphorus can be removed from wetlands if the plant biomass is harvested (every year). The plant biomass can then be composted safely.

Typical applications

Rainwater treatment: wetlands can be efficient as part of a remediation process to for the treatment of rainwater that is contaminated due to runn off or atmospheric deposition (e.g. too much nutrients, pharmaceuticals, oil, petroleum and heavy metals).

Domestic waste watertreatment, industrial waste watertreatment: Water from the sewage system and from industry can be successfully treated with biotreatment system. Contaminants that can be removed are nutrients, organic substances, heavy metals, etc.

Agriculture and horticulture sector: The largest source of surface water contaminants with crop protection agents in Flanders are point source contaminations (40-90%) (PCfruit purification systems brochure). The contamination occurs during the filling and cleaning of spraying equipment: spillage, overflowing of spraying machines during filling, leaking of pipes or the discharge of spray residues and rinsing and cleaning water. Farmers are often not aware of the large amount of water contaminated with plant protection products, or they fear unjustified additional high costs, work and obligations to counter this problem. In addition to preventive measures to prevent the problem, collected ‘residual’ water can be treated *at the site* using constructed wetlands or biotreatment systems. There are two types that are widely used in Flanders, namely the biofilter and the phytobox (PCfruit purification systems brochure).

Biofilter: A biofilter consists of two parts: the first part is the filter unit and comprises three stacked containers with a capacity of 1 m^3 (= cubitainer). The second part is the evaporation unit and comprises multiple containers with plants. The function of the filter unit is to treat the contaminated water. The stacked cubitainers are filled with substrate in which microorganisms live. When water flows through the system, a large proportion of the contaminants will be adsorbed, this adsorption will give microorganisms time to absorb the substances and degrade them at the same time. An efficient mixture for a filter unit is 50% chopped straw, 40% compost and 10% vegetable mold. The evaporation unit of the biofilter increases the evaporation capacity of the system. The cubitainers of the evaporation unit can be filled with a mixture of planter soil 90% and 10% vegetable mold. Sedges (*Carex* spp.) and willows (*Salix* spp.) are often planted, as they can evaporate large amounts of water. In addition, based on visually observable symptoms of phytotoxicity, the plants can act as an indicator of the contaminant load of the system. A biofilter with 3 vertical substrate containers and 3 plant containers can evaporate a maximum of 5 m^3 of residual water on an annual basis. If evaporation is not chosen then a similar volume of water can be used to irrigate. (See **Figure 23**).

Figure 23: Biofilters in practice. Photo: PCfruit test centre, Sint-Truiden.

Phytobassin: Another type of biotreatment system is the phytobassin. This is a container filled with a substrate mixture with microorganisms to degrade the contaminants. This bassin is planted with willow or sedge. The water from the bassin evaporates. An efficient filling mixture is 50% chopped straw, 40% compost and 10% vegetable mold, other examples use 70% soil and 30% straw. To prevent rainwater from entering the phytobassin, a roof construction is installed. This construction can also stimulate evaporation. Special nozzles or a small drip hose ensure that the residual water is evenly distributed over the phytobassin. (See also **Figure 24**).

Figure 24: Phytoboxing in practice Source: PCfruit Sint-Truiden test centre.

Plant selection

Plants in reed beds, biofilters and phytobassins are usually chosen to remove as many contaminants as possible. Species with a high evapotranspiration rate are interesting for phytobassins if evaporation is chosen instead of reusing the treated water.

Wet zones can be partially planted in the most contaminated zones with species such as Small cattail (*Typha angustifolia*), Common cattail (*Lythrum salicaria*) and Yellow iris (*Iris pseudacorus*). The selection of the species is dependent on the type of the contamination. Plants such as Reed (*Phragmites australis*) can be planted at the water table. Wet zones where less contamination is present are sown with specific species for wet or marshy soils.

4.3 CHOICE OF THE SUCCESSION STAGE ACCORDING (COREMANS *ET AL.*,2011)

Pioneer vegetation is the vegetation on bare and disturbed soil. They are mainly annual plants that form many and light seeds and can spread quickly. Among the pioneer plants are also a lot of unwanted herbaceous plants such as Peach herb (*Persicaria maculosa*) and Goose Foot (*Chenopodium album*), but also varieties such as Cornflower (*Centaurea cyanus*), Large Poppy (*Papaver rhoeas*) and Real Chamomile (*Matricaria chamomilla*).

After a while, usually after just one growing season, a pioneer vegetation evolves into a grassland vegetation. Grasses are perennial, germs slower and roots deeper than pioneer plants. The grass roots form a mat that is impenetrable to the roots of pioneer plants with the result that they disappear. In addition to grasses, grassland plants also occur. They have deep roots and often a rosette that casts shadow on the grass to keep the grass at a distance. They also have long flowering stems to stand out above the grasses. A number of well-known species are Marguerite (*Leucanthemum vulgare*), Yarrow (*Achillea millefolium*) and Long-leafed honorary prize (*Veronica longifolia*).

If we do not mow the grass or have it grazed, it will blossom and the culms will lay flat, creating a pack of stacked grass with the lower layer slowly digested. A rough herb vegetation then settles, high herbs with strongly developed roots that grow on nutrient rich soils. This group includes Large Nettle (*Urtica dioica*), Blackberries (*Rubus* sp.), Liverwort (*Eupatorium cannabinum*), Hairy Willow-Herb (*Epilobium hirsutum*), Tansy (*Tanacetum vulgare*), Meadowsweet (*Filipendula ulmaria*) and Cattail (*Lythrum salicaria*). Grass species and grassland plants are slowly disappearing.

Seedlings of shrubs and trees pop up. Their seeds are spread by wind, water and animals. Shrubs appear, such as Hawthorn (*Crataegus* sp.) and Elder (*Sambucus nigra*) and the first trees such as birch, alder and willow. Over time the trees grow and due to a lack of light a number of shrubs, grasses and rough herbs disappear. The thicket slowly turns into a forest.

If we want to maintain a certain succession stage, we must intervene. Otherwise everything develops to a forest. To prevent a pioneering stage from turning into grassland, we must manage the land. To prevent a grassland from turning into scrubland, we have to mow at least once a year. To prevent scrubland from turning into forest vegetation, we have to mow at least every three to five years.

The choice of the succession stage therefore largely determines the view and especially the management. That is why a well-considered choice, regardless of the specific plant choice, is crucial for the further approach and for the current and future use of the site.

When applying annuals (pioneer vegetation) it may be necessary to till the soil each year by e.g. milling. This can increase management costs and increase the risk of spreading contaminated soil particles via wind or water erosion.

An important factor in applying phytoremediation is that the contamination is accessible to the roots of the plant. This means that we use deep rooting shrubs and trees for deeper contamination. For example, phreatophytes can develop roots into the groundwater table.

In many cases it is valuable to have different sub-concepts with different succession stages matched up.

The succession stages are translated into the following forms and typologies (as discussed earlier) that are applicable within phytoremediation:

- Pioneer vegetation: flower field, annuals used as extraction cover, degradation cover, stabilization cover;
- Grassland vegetation: lawn, flower meadow, bulb grassland, grassland, grasses and/or grassland plants used as extraction cover, degradation cover, stabilization cover, extraction border, degradation border, stabilization border;
- Rough vegetation: perennial herbaceous plants used as extraction cover, degradation cover, stabilization cover, extraction border, degradation border, stabilization border;
- Forest vegetation: hedge, bush, wood edge, forest used as extraction cover, degradation cover, stabilization cover or hydraulic barrier.

4.4 MANAGEMENT

Because phytoremediation uses living organisms, specific management requirements compared to other more traditional remediation systems are necessary:
Maintaining a healthy system is crucial for the continuation and effectiveness of the remediation process. Variations in plants, climates and contaminants can give rise to some, all or additional requirements such as:

- Visual inspections
- Fertilization
- Irrigation
- Management of unwanted herb growth
- Mowing
- Felling/pruning
- Disposal of biomass
- Protection against damage
- Replanting

Visual inspections, fertilization, irrigation and protection against animal damaga are measures to protect the plant and to control or stimulate growth. Management of unwanted herb growth helps with both plant growth and with the prevention of invasive species.

Mowing is primarily implemented to maintain grassland and rough vegetation, to manage unwanted herb growth or to keep the site accessible in specific cases.

Felling/pruning may be necessary as a control measure depending on the shape of the plants (Short Rotation Forestry, pollard tree, etc.). However, if the contaminants have accumulated in the plant tissue, the soil becomes remediated provided that the biomass is removed. If the concentration of contaminants exceeds the applicable standards, the biomass must be disposed of as hazardous waste (VLAREMA) at extra costs. In special cases, some contaminants that have accumulated in the plant tissue, such as heavy metals, can be recovered and sold. This is known as phytomining. In such cases, these “cash crops” can be an asset to the project by reducing part of the total cost.

Protection against eating by animals is important not only to protect planting but also to prevent contamination spreading through the food cycle in specific cases.

Replanting may be necessary to maintain plant density to guarantee a continuous uptake of contaminants. Vegetation can die for various reasons (i.e. damage from animals, insects and the weather) and must then be replanted in function of the maintenance of the root mass required for the uptake of contaminants and the release of exudates.

Dead plant material and pruning waste, if it contains no contaminants, can be composted on site or processed. The presence of dead wood will in many cases provide an ecological added value for the site.
Frequent site visits and maintenance during the first year of a planting are crucial and play a major role in whether or not phytoremediation will succeed. The availability of moisture and management of unwanted herb growth are some of the more critical requirements (Compton, 2003).

The choice of the desired succession stage is also important in function of future management as well as the current and future use of the site or the various sub-sites.

Succession refers to the natural evolution of an fallow field to a vegetation with pioneer plants, then grasses and grassland plants followed by tall forbs and finally scrub and forest (Coremans *et al*. 2011).

4.4.1 Pioneer stage

Type of greenery	Management
Flower field or specific annuals	Mow once a year and remove cuttings + annual tillage of the soil

Annuals are mowed once a year in October. The cuttings are always removed to prevent overgrowth. Subsequently tillage of the soil is necessary, for example by raking or milling. That is why permanent coverage with annuals is not always advisable on contaminated soil. The tillage of the soil can entail a risk of spreading contaminated soil particles.
If necessary, the tillage of the soil can be combined with the sowing of extra annuals.

The cuttings are removed from the site or can, for example, be composted at the site. The actual processing of the cuttings depends on the potential stored contamination in the biomass (see below).

4.4.2 Grassland

Type of greenery	Management
Lawn	Mow 20 times per year
Flower meadow/ extensively managed grassland	Mow 1 to 5 times a year and remove cuttings
Flower meadow	Weed 3 to 5 times per year Mow 1 or 2 times per year and

	remove cuttings

Where necessary (hiking trails, lawns, play areas, etc.), intensive management is conducted. Where possible, grassland is extensively managed as much as possible. Flower meadows are mowed once or twice a year depending on the food richness. The mowing time is chosen according to the flowering (often a first flowering ends at the beginning of June and a second at the end of August).

Weeding is limited to the possible removal of unwanted herbaceous plants. Tillage of soil (raking, etc.) is never carried out because this stimulates the growth of unwanted annual herbs.

The cuttings are removed off site or can, for example, be composted on site. The actual processing of the cuttings depends on the potential stored contamination in the biomass (see below).

4.4.3 Scrubland

Type of greenery	Management
Flower meadow Specific perennials	Weed 3 to 5 times per year Mow 1 or 2 times per year and remove cuttings In some cases, lower mowing frequency to 1 time every 3 years.

Scrubland can be mowed at a lower frequency depending on the food richness and the desired species. Weeding is limited to the possible removal of unwanted herbaceous plants. The tillage of the land (raking, etc.) is never carried out because this stimulates the growth of unwanted annual herbs.

The cuttings are removed from the site or can, for example, be composted at the site. The possible processing of the cuttings depends on the potential stored contamination in the biomass (see below).

A specific situation is the management of wet zones with bank plants.

Most herbaceous vegetation can be mowed annually, removing the cuttings. The zones with Yellow Iris should be mowed as little as possible. Mowing can cause Yellow Iris to disappear. The zones with Reed can be mowed in early March.

4.4.4 Forest

Type of greenery	Management
Herbaceous vegetation with shade plants	Weed 1 to 2 times per year
Woodland	Remove seedlings of trees and bushes
Woodland edge / shrub belt	Remove seedlings of trees and bushes
Climbing plants	Little - much pruning depending on the species
Hedge	Prune 1 to 3 times per year
Hedgerow	No or sporadic pruning
Cut wood	Fell 1 time every 5 to 10 years
Pollard tree	Pollard 1 time every 5 to 10 years

The released pruned material and wood can remain at the site as dead wood, for example processed in a branch walls or can be removed for further processing depending on the type and the amount of contaminants stored in the biomass (see below).

In the first years after planting, it is necessary to exempt the trees once or twice a year so that they do not get overgrown by grasses and/or herbaceous plants.

4.4.5 Processing of biomass

A final destination of the biomass is selected according to the applicable standards.

In Flanders the VLAREMA should be used concerning the sustainable management of material recycling and waste.
- If the concentration does not exceed the applicable standards, the biomass can be removed for aerobic or anaerobic processing.
- If the concentration of the contamination exceeds the applicable VLAREMA standards, the biomass must be disposed of for incineration.

4.4.6 Fauna friendly management

Green zones have a special ecological function. Fauna friendly management of these zones increases this ecological value.

In addition to pesticide free management, the following simple actions contribute to fauna friendly management:
- Mow grassland vegetation in a limited way so to prevent disturbing the life cycle. Do not mow about 1/5 of the grassland.
- Provide gradual transitions between different sub-concepts. This provides additional variation in structure.

- Mow grassland plants no later than September.
- Avoid grass pollen in grassland zones with extensive management.
- Do not mow shorter than 6 cm.
- Keep sufficient dead wood at the site. For this purpose, pruning material can be stacked, for example, in branch walls.

4.5 MONITORING (DESIGN)

The phytoremediation system must be periodically monitored to evaluate the evolution and efficiency of the end result. The monitoring depends on the phytoremediation technique and the chosen remediation objective.

In particular, it must be evaluated whether the contamination and/or contamination plume is stable or shrinking. A well-designed monitoring system leads to a database with trend data of the relevant parameters over time and space. The collection of relevant data makes it possible to determine changes in background concentrations and in the groundwater flow.

The monitoring plan must at least meet the following requirements:

1. demonstrate that the phytoremediation technique proceeds as predicted;
2. identify all toxic by-products that may be formed;
3. determine whether the contamination or contamination plume is stable or shrinking;
4. determine that no sensitive receptors are threatened;
5. discover changes in environmental conditions that may compromise the effectiveness of the phytoremediation technology;
6. verify the achievement of the remediation objectives

A monitoring program is always site-specific. All available data from the preliminary survey (investigations and site characterization) can be used to prepare a conceptual site model and a monitoring plan.
A sampling and analysis program must be drawn up, including:

- the location of the sampling points;
- the parameters to be determined, possible degradation products and the method of sampling and the medium to be sampled (soil, groundwater, soil air, plant);
- the periodicity of monitoring (sampling frequency) and
- duration of the monitoring.

4.5.1 Monitoring locations

The location of the sampling points depends on the medium of the contamination:

- In case of soil contamination, soil samples are taken in relation to the depth and presence of the contamination.
- In case of groundwater contamination, monitoring wells are installed. The purpose of these monitoring wells is twofold: to evaluate whether the behaviour of the contamination in the groundwater changes and to evaluate whether the contamination in the groundwater is stable/shrinking. The monitoring wells installed to observe the behaviour of the groundwater contamination must be installed at least in the following zones, depending on the mechanism:
    1. upstream of the source of the contamination in the non-contaminated zone to control the upstream groundwater quality;

2. in the source of contamination, if this zone is remediated with a phytotechnology, to estimate the change in the concentration over time. Sufficient monitoring wells must be installed to be able to estimate the decrease in source zone concentrations;
3. phytohydraulics: downstream of the contamination, in the direction of the groundwater flow, to monitor changes in behaviour and concentrations of contamination; the number of wells depends on the size and width of the plume;
4. alarm monitoring wells at critical zones such as, for example, at the plot boundaries downstream the contaminated groundwater plume. These wells are essential to obtain timely insight into any spreading of the contaminated plume and thus being able to proceed in time with alternative containment measures to prevent further spreading to, for example, a sensitive receptor;
5. Lateral: to monitor lateral spreading of the groundwater contamination.

4.5.2 Monitoring parameters

General parameters that can be monitored during phytoremediation are summarized in **Table 11**. Depending on the individual phytoremediation system, it must be decided which parameters should be included in the monitoring plan. **Table 11** can be used as a toolbox.

4.5.2.1 Climatic data

To monitor the water balance of the site and the evapotranspiration of the plant, it is important to monitor the climate data. At least the temperature, rain fall, relative humidity, sunshine, wind speed and wind direction are taken into account.

4.5.2.2 Plants

The following characteristics can be monitored for the plant:

- visual properties (health, stress, damage by animals, leaf mass, etc.)
- (evapo)transpiration and quantification of degradation products in the various plant tissues (roots, stems and leaves).

Visual evaluation

For the visual inspection of the condition of the plant, one can initially evaluate the presence of leaves (mass), possible leaf discolouration, branching pattern, retarded growth, reaction to wounds, the extent to which wound overgrowth tissue is formed and symptoms that indicate damage by insects, bacteria, viruses or fungi. These assessment keys for damage and measuring instruments are tools that allow you to assess plants in a standardized way.
Still, every plant, location and context is different and there are many factors such as effects due to wind that must be taken into account. There are also other methods such as length measurement of branch shoots, biomass determinations, growth ring analysis, and starch analysis that can help in obtaining a more complete picture of the condition of the plant.
During a growing season there are also various methods for measuring the growth and condition of the vegetation. The condition can be observed with a number of sensors, and one can also get an impression with these sensors of the variation in vegetation development within a plot. The measurements are based on vegetation reflection and calculate a vegetation index. It is also assumed that the amount of biomass and the colour of the crop are an indication about the vitality. Stress factors such as water and nutrient deficiencies can therefore be made visible. There are "Near Sensing Systems" that measure directly above the vegetation, or "Remote Sensing Systems"; measuring from a distance above the vegetation, for example with satellites or UAS.

Evapotranspiration and quantification of degradation parameters

If phytodegradation is applied (whether or not microorganism assisted), it is important to confirm that the uptake of contamination (and any degradation products) by the plant does not result in the volatilization through the leaves to the air.

Measurements of volatilization through the leaves are not easy. Under controlled laboratory conditions, volatilization can be measured directly at the leaves (see 3.4.1.4). However, this is practically difficult to do for large-scale *in situ* phytoremediation systems.

Another method that can be used to measure whether the contamination does not transpire to the air through the leaves, is the measurement of the concentrations of organic contamination in the sap flow. The concentration is determined at different heights in the stem. It is assumed that if the concentration of organic contamination at the top of the stem, close to the leaves, has decreased sufficiently, little or no transpiration is occurring through the leaves.
The concentrations of water soluble organic contaminants and potential degradation products are measured via small drillings in the stem, and the extraction of metabolites in the sap flow via solid-phase micro-extraction (SPME) or solid-phase spectrophotometry (SPS) and analysis on site or in the lab (Burken *et al*., 2011) (**Figure 25**).

Figure 25: Drilling down to sap flow and SPME or SPS sampling. Source: Joel Burken lab, Missouri S&T, USA.

This method can be used to compare the evapotranspiration of trees that are inoculated with **VOC-degrading bacteria** or non-inoculated trees. To optimize the remediation process, additional inoculations can be performed in a timely manner.

When choosing a plant for phytoremediation, we take into account the possible risks of **spreading the contamination via the food chain**. For example, in certain cases it may be advisable not to use agricultural and horticultural crops. In the case of phytoextraction, leaf fall can pose a risk of spreading.

The uptake and accumulation of metals in plant tissues requires monitoring of the concentration of metals in the (possibly consumable) plant tissues (leaves, seeds) to evaluate bioaccumulation of the contaminant and to evaluate the risk of transfer to the food chain. In the monitoring program leaf, shoot, seeds should be collected, extracted with concentrated acid and analysed with ICP-OES. These monitoring schemes can also be used to identify whether inoculations with tolerant fungi or bacteria are necessary to, for example, stimulate stabilization of metals in the roots instead of translocation to edible parts of plants.

4.5.2.3 Soil

If the phytoremediation technology is aimed at stabilizing, extracting, and concentrating contaminants in the soil, the efficiency of the technique should be evaluated by taking soil samples. The soil samples should be analysed for the relevant parameters in an accredited laboratory.

The parameters to be analysed in the soil sample are at least the contamination parameters and degradation products for which a remediation need is defined.

4.5.2.4 (Ground) water

If the phytoremediation technology is aimed at the remediation or containment of groundwater, the following parameters should be monitored:

- groundwater sampling and analysis for the relevant parameters in an accredited laboratory. The groundwater samples should be analysed at least for the contamination parameters and degradation products for which a remediation need has been defined.
- the local groundwater flow direction and velocity.

If **soil additives** were added to increase the bioavailability of metals, groundwater monitoring is necessary to monitor possible leaching of the metals. Groundwater samples should be taken and analysed for the relevant parameters.

4.5.3 Periodicity and duration of monitoring

The periodicity and duration of monitoring depends on the technique and the proposed remediation objective.

Table 11 gives an overview of the monitoring parameters and the frequency. This table provides guidance and must be further detailed and worked out by the soil remediation expert.
When determining the sampling frequency in the case of phytohydraulics, the following must be taken into account:

- Spreading velocity of the contamination: the **sampling frequency** must be adapted to evaluate the spreading of the contaminated plume towards any nearby receptors.Seasonal changes in local hydrology are also important in the evaluation. The intervals between successive monitoring campaigns may not exceed the time required for the contaminated plume to reach the first non-contaminated monitoring well.

In general, the frequency of plant monitoring is parallel to the age of the plant; for example, sampling can be carried out at least annually if it is not seasonally relevant or possible.
From the precautionary principle, the intervals between the initial campaigns can be higher, for example half-yearly (once in summer and once in winter) for the first two or three years and annually thereafter. A higher monitoring frequency may be required for highly mobile contaminants. Lower frequencies can be chosen if the contamination is less mobile. The **monitoring period** is at least the time required to achieve the proposed remediation objectives.

The monitoring frequency can be reduced in function of the results obtained and with in depth motivation. All this is clearly shown in **Table 11**.

Table 11: Summary of monitoring parameters, reason for monitoring and measurement frequency

Monitoring parameter	Reason for monitoring	Frequency (recommended)
Climatological data		
Temperature	- Maintenance in function of type of plants (irrigation, etc.)	Seasonal
Rainfall	-To determine the water balance and velocity of evapotranspiration	
Relative humidity		
Sunshine		
Wind speed and wind direction		
Plants		
Visual characteristics (signs of stress, disease, vitality, damage from insects, fungal disease, growth, leaf mass, etc.	- Maintenance in function of type of plants: replanting, replacement of plants, fertilizing, pesticide use	Trimester or seasonal
Tissue conc. (root, shoot, stem, leaf)	- Determine contaminant and degradation products	Annual
Transpiration velocity/VOC evapotr.	- Quantification and evolution of the phytoremediation process	First time two years after planting, then possibly every two years
Soil/sediment		
Geochemical parameters (pH, nutrient concentrations, water content, etc.)	- Optimization of characteristics for vegetation	Seasonal/annual
Microbial communities	- Quantification of degradation potential	Annual (afterwards every two or three years)
Contaminant and degradation products	- Quantification and evolution of the phytoremediation process	Annual
(Ground)water		
Groundwater characteristics (Groundwater flow velocity, depth, etc.)	-Quantification of contaminant and concentrations of degradation products	Seasonal/Annual
Contaminant and degradation products	- Quantification and evolution of the phytoremediation process	Seasonal/Annual

4.5.4 Evaluation of the monitoring data and optimization of the monitoring program

The monitoring program is an iterative process in which the results are evaluated after each monitoring campaign to determine whether the results confirm the conceptual site model (**Figure 26**).

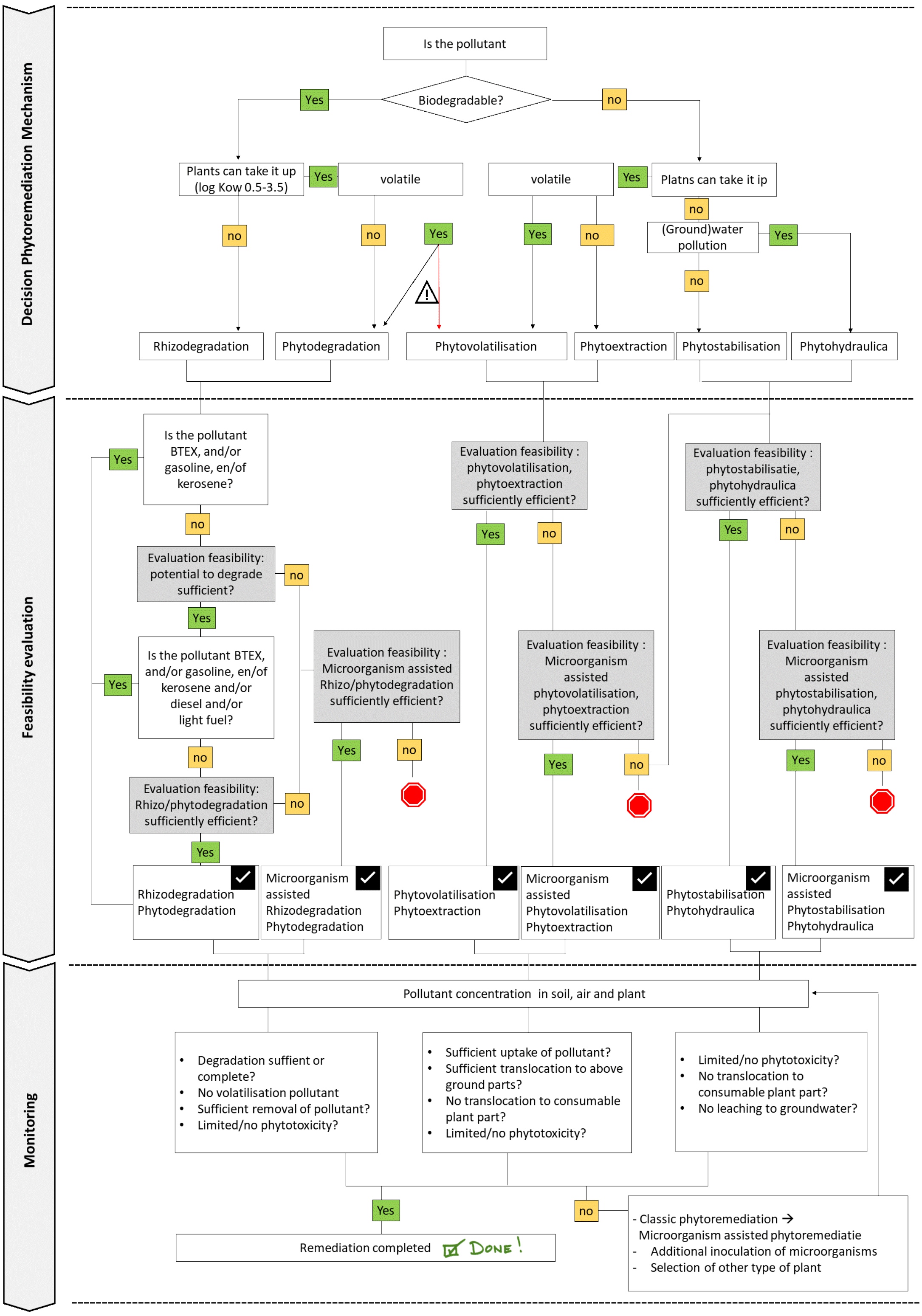


Figure 26: Decision tree: Monitoring.

If the results confirm the conceptual site model and the evolution proceed as expected, the intensity of the monitoring campaign can be reduced.
If it appears that the chosen phytoremediation technology does not proceed as expected, the cause of this discrepancy must be carefully considered (wrong assumptions in the conceptual model? Or concentration data not correct or geochemical background data not correct?). In this case it may be appropriate to intensify the monitoring program or to perform additional tests in the laboratory.

Phytoremediation is an innovative technology. Therefore, an essential part of every phytoremediation monitoring program is an action plan that describes the need for active measures when monitoring activities show that phytoremediation is not proceeding according to expectations (**Figure 26**). To evaluate the performance of phytoremediation, the remediation objective are taken into account. On the one hand it can be a contaminant mass reduction or mass removal and on the other hand a phytostabilization. In both cases, the concentrations of the contaminant and degradation products in the soil and/or the (ground) water must be measured and the evolution should be monitored.

The result of the monitoring must be described in an interim remediation report to OVAM. If the evolution of the results is not sufficiently favourably, it is advisable to perform additional measurements to determine whether optimization of the technology is possible:

- counting the bacteria in the rhizosphere or in the groundwater, for example; the microbial potential to degrade the contamination (present genes) can be quantified using molecular DNA techniques (see also 3.2.2.3 laboratory and field tests).
- installing additional monitoring wells to become a better picture of the contaminant evolution and/or potential influx of contamination.
- Sampling/analyzing soil conditions to determine whether additional additives are necessary.

In general, if classical phytoremediation does not achieve the remediation objectives, microorganism assisted phytoremediation can be considered. If the expectations of microorganism assisted phytoremediation are not met, it may be considered to make additional inoculations or to review the plant choice. If the above actions do not provide the necessary improvement in case of phytoextraction and phytovolatilization, reconsideration of the phytoremediation mechanism (phytostabilization or phytohydraulics) may offer a solution.

In any case, one should take into account the time required for the plants to reach the groundwater and/or contamination and hence conclusions must not be drawn too quickly.

If optimization of the phytoremediation is not possible, an alternative or additional remediation concept or technology can be implemented.

4.6 COST ESTIMATE

The cost estimate for remediation with phytoremediation takes into account four main categories of costs: (1) Design, (2) Setup (3) Management and (‘)Sampling and analysis (see also Section 2.7.2).

(1) When designing a phytotechnology (Section 4.1), the tests that are required to design the full scale remediation in practice must be taken into account. In this context, particular attention should be given to the feasibility tests that may be required and/or the plant selection tests of Section 3.4 (In depth evaluation of feasibility). An overview of the associated costs can be found in Table 8. In addition, costs of the associated engineering for the preparation of the tender specifications could be included.

(2) For the setup of phytooremediation, the preparation of the location, the soil, the planting material, as well as any necessary irrigation or damage protection materials should be taken into account. More detail is given in section 4.2. The costs for soil tillage for phytotechnologies are comparable to those for agriculture, horticulture or forestry. These costs are generally between €300 and €400/ha. However, depending on various parameters, this can amount to more than €100.000/ha.
For example, the following must be taken into account:

- The preparation of the site: € 0,5 - 100 and more per m^2;
- Planting (including planting material, piles, damage protection and drainage pipe): € 1 - 40 per m^2

(3) The management costs include all necessary costs associated with the management of the site, the planting, the possible harvest and the phytotechnology set up (including bioaugmentation) over the entire remediation period. The monitoring wells, power sources, maintenance of the equipment and labour are also included. Specific management requirements for phytoremediation are described in Section 4.4 of this document. The management costs can also vary from a few hundred euros to more than €100.000/ha over the entire remediation period. For example, the following must be taken into account:

- the required mowing or tillage between the plantings: 0,1 - a few € per m^2;
- maintenance pruning, pollarding and possibly harvesting: up to a few € per m^2;
- irrigation: up to a few € per m^2
- bioaugmentation (if necessary): a few to tens of € per m^2.

(4) Monitoring the efficiency and result of the remediation consists of the sampling and analysis costs. These costs can dominate the total cost of the project due to the time required for monitoring and the required amount of data. The costs mainly include sampling time (performance of soil drilling and groundwater sampling) and laboratory costs for analysing the samples. Data collection during sampling and analysis are crucial for the in depth documentation of the progress and the performance of phytoremediation as a new technology.
Monitoring parameters and frequency are shown in Section 4.5.3.
The monitoring of volatilization must be taken into account. These costs can amount to more than €10.000 per campaign, depending on the number of plants to be monitored.

4.7 LEGISLATIVE FRAMEWORK

When setting up a phytoremediation project, specific legislation that applies to the site must be taken into account. In Flanders, the necessary permits are applied for by submitting the soil remediation project. After all, the chances of success of the soil remediation project will greatly increase if the feasibility study and the design and management take maximum account of this legislation.

The Rural Code (veldwetboek) of October 7, 1886 regulates, among other things, the **distance of plants** from the property boundary. The provision of article 35§5 is also important: in the parts of the site intended for agriculture and horticulture, forestation is prohibited at less than 6 metres from the property boundary. However, planting of linear landscape elements is not considered to be forest planting. Moreover, forest planting in agriculture and horticulture requires a permit from the mayor and aldermen's college. These provisions also apply to areas for forestry adjacent to those for agriculture and horticulture.

The Forest Decree (bosdecreet) of 1990 regulates the various **forest functions**. For most forests, these are specified in a forest management plan. The criteria for sustainable forest management were added in 1999.

Actions that were not included in a forest management plan are subject to authorization from the Agency for Nature and Forests.

Whether a group of trees is **defined as a forest** is determined by the **presence of trees or shrubs on the one hand and the presence of a forest's own fauna and flora on the other**. The spatial destination plays no role in determining whether vegetation is defined as a forest. The area also plays no role!

The following vegetation is never considered to be forest in Flanders:
- ornamental plants and gardens;
- short rotation forestry (SRF) whose planting took place on land located outside the spatially sensitive areas. SRF is a cultivation of fast-growing woody crops that are harvested with very short cycle times (max. 8 years). Plantations of, for example, poplar and willow with a turnaround time of 2 to 5 years and intended for energy extraction or plantations with the aim of producing fibre for paper or producing biomass with a turnaround time of up to 8 years are not considered to be forest.
- crop growing whose aboveground mass is harvested in its entirety periodically up to three years after planting or after the previous harvest. The rotations are very short and the main objective is the production of wicker.

The following vegetation is **always** considered to be forest in Flanders:
- plantations that are mainly intended for the wood yield, including those of poplar and willow, with the exception of the short rotation forestry (SRF) on land located outside the spatially sensitive areas. Consequently, homogeneous plantations of cultivated poplars are forest unless they are also used as agricultural and horticultural land (pasture or hay meadow) and can be considered to be a forest farming system (agroforestry);
- willow, with the exception of wicker cultivation with short rotations and production as the main objective;
- coppice cultures: coppice is a form of forest management and is therefore considered to be forest;
- thickets, where we only find woody shrubbery vegetation and no trees, are considered to be forest.

A plot with trees, for example **a meadow**, can in certain cases be considered to be forest. The criterion used to determine this is the coverage ratio. This is the ratio of the total area of all crown projections to the total forest area. If it is larger than 50%, projected to full-grown stage, it is forest. With a cover ratio < 50%, there is open vegetation with or without scattered trees (e.g. heathland with flies) (no forest), or an open space within the forest (which is considered to be forest if it is a max. of 3 hectares in size and half surrounded by forest).

The Nature Decree (Decree of 21 October 1997 concerning nature conservation and the natural environment, amended by the Decree of 19 July 2002). A **nature permit** is required for, among other things, changing vegetation and small landscape elements in a series of spatial destinations and in areas with a certain protected status, unless the necessary works are already provided for in an approved management plan, or if this is stated in another permit (for example an urban development permit) and if the Agency for Nature and Forests has recommended this permit application. It is therefore advisable to request this recommendation explicitly from the government that must issue the permit. In most cases that will be the municipality. If a (felling) permit has been obtained from the Agency for Nature and Forests according to the Forest Decree, a nature permit is no longer required. A nature permit is also not necessary in the immediate vicinity of a licensed home or industrial building (100 metres or 50 metres in a ground area, park area, buffer area or forest area). Furthermore, no nature permit is required for normal maintenance of vegetation. For additional information see permits and small landscape elements.

The Species Decree of 15 May 2009 determines which species of animals and plants are protected in the Flemish Region (Article 9), and what legal consequences are associated with that protected status (Article 10-

18). In the first place, a series of actions is mentioned that are prohibited with regard to protected species. More information can be found on protected species.

The Landscapes Decree of 16 April 1996 and associated implementing decrees. This legislation applies to sites that are located in protected landscapes.

The **2004 EIR decree**. Among other things, an environmental impact report (EIR) must be drawn up for afforestations of more than 10 ha and for deforestations of more than 3 ha.
In addition, there are also numerous **subsidy possibilities** for carrying out afforestation or reforestation and for promoting ecological forest functions.

Table 12: Overview of the most important legislation in the context of phytoremediation.

Legislation	**Motivation**
Soil remediation decree and VLAREBO	Phytoremediation technology, soil remediation
Forest Decree - outside sensitive areas	A planting with tree-like plants is never considered to be a forest provided that it is harvested within 8 years.
Forest Decree - within sensitive area	In certain cases, the forest compensation obligation must be taken into account. In the context of the soil remediation project, an exemption from the prohibition on deforestation may be obtained through the Flemish government.
Landscape Decree	For planting tree-like plants, it is necessary to request a permit under the Landscape Decree from the Property Heritage Agency.
Rural Code	When planting trees, a distance of 2 m to the plot boundary must always be maintained.
Materials Decree and VLAREMA	For the removal and processing of the biomass.
Environmental permit decree and environmental permit decree	Environmental permit – felling permit for planting with tree species.

5 PROCEDURE FOR CLOSING STRATEGY AND AFTERCARE

5.1 STARTING POINT

The remediation can only be regarded as final after the remediation objectives have been achieved, as described in the soil remediation project or after mutual consultation with OVAM. All data must be included in a final evaluation study.

5.2 EFFECTIVENESS AND EFFICIENCY OF THE REMEDIATION

During remediation, the soil remediation expert must evaluate whether the efficiency of the phytoremediation (technology) is sufficiently high and whether optimization is necessary. In case of insufficiently effective remediation by the chosen phytoremediation technology, the remediation design may need to be optimizes to achieve the proposed remediation objectives. The soil remediation expert must indicate to the client and OVAM, respectively, if the remediation is not effective and efficient enough.

Some phytoremediation technologies (phytostabilization and phytohydraulics) are containment strategies. In other words, this concerns long-term remediation where no specific remediation values are proposed. The objective is, after all, to prevent the spreading of the contamination. It can be argued that with these remediation technologies, remediation can be considered as final when there is no longer a risk or a stable final condition is achieved.

5.3 FINAL EVALUATION STUDY OF THE REMEDIATION

If the results of the remediation show that the remediation objective has been achieved, or an equilibrium/stable situation has been achieved, or in case of sufficient reason why the remediation can be ended, a final evaluation report of the remediation will be drawn up. This report must provide a clear overview of the soil remediation work carried out (implementation of phytoremediation technology) and the evolution in the concentrations from the initial implementation phase. It must be clearly indicated whether it is desirable to remove the vegetation or whether it can remain on site.

The monitoring of the soil and/or groundwater upon completion of the remediation work must take place after the equilibrium situation in the soil has been reached.

In the case of groundwater contamination, the efficiency of the remediation should be evaluated by at least the downstream monitoring wells and the monitoring wells in the source zone of the contamination with the highest concentration of contaminants.

In the case of a contamination in soil, the efficiency of the remediation should be evaluated by at least a minimum of soil samples at the source zone of the contamination with the highest concentrations.

A final evaluation study must be submitted to OVAM, taking into account the standard procedure for soil remediation work and aftercare.

5.4 PROCEDURE FOR CLOSING STRATEGY

The soil remediation works can be considered to be finished if:

- the remediation objectives have been achieved, in accordance with the conditions in the certificate of conformity and taking into account the BATNEEC principle;
- a stable situation is achieved. The final state is confirmed by monitoring.

If those conditions are met, a final evaluation study can be submitted to OVAM. This study must contain a description of the work performed and the results of the soil remediation.

When the crops are removed after remediation, the conditions included in the soil remediation project must be taken into account for the processing of the contaminated biomass.

If phytoremediation is used as a control (phytohydraulics) or if long term phytoextraction is applied and the plants cannot/may not be removed, the remediation can be considered as final and “advices for use of the site” will be included for the maintenance of the plants.

When the soil remediation work has been completed, aftercare is provided or “advices for use of the site” is formulated (see standard procedure for descriptive soil investigation and soil remediation project).

5.5 AFTERCARE

Aftercare includes measures to evaluate of the quality of the soil and measures based on the maintenance and proper functioning of the “remediation infrastructure”.
Examples of measures as a function of the maintenance and proper functioning of the remediation infrastructure in phytoremediation are, for example:

- Control and maintenance of the vegetation/plants;
- Maintenance of the fences;
- Maintenance of the groundwater table (e.g. permanent decrease of the groundwater table);
- Possible removal and/or processing of biomass

When the aftercare has been completed, “advices for use of the site” is formulated (see standard procedure for descriptive soil investigation and soil remediation project).

6 SAFETY AND HEALTH ASPECTS

As for other remediation technologies, safety and health aspects are also important during the implementation of the phytoremediation.

When drawing up the soil remediation project, a risk analysis must be made for the chosen phytoremediation technique. All safety and health risks must be included in this risk analysis. “Health and safety” starts with good design and project planning. Safety therefore starts in the soil remediation project. After the declaration of conformity of the soil remediation project, the soil remediation works will be carried out.

Care for safety, health and the environment play an important role in implementing soil remediation work. This quality has been guaranteed for years by the Achilles system (in Flanders). Every soil remediation must be applied in accordance with the Achilles care system as included in the standard procedure for “soil remediation works, final evaluation study and aftercare”.

The Royal Decree applies to temporary and mobile workplaces when carrying out soil remediation works. In other words, a safety coordinator is appointed for soil remediation work. The appointed safety coordinator and the soil remediation expert will impose specific safety measures when implementing the remediation. These must be implemented by the remediation contractor.

The potential safety and health aspects in conducting the pilot tests and remediation with phytoremediation technologies are related to the soil contamination at the site itself, the contamination taken up by the plant and the contamination leaving the plant through volatilization. Subsequently, the safety and health aspects must be taken into account during the execution of the works (digging, planting, sowing, etc.). Many of these risks can be prevented by good management and personal protective equipment (PPE). These PPE are dependent on the type and concentration of the contamination. The standard PPE include a helmet, a safety jacket, safety shoes and safety gloves.
Depending on the contamination, these standard PPE must be supplemented with additional personal protective equipment. For example, respiratory protection (e.g. full-face mask or half-face mask) is used for volatile contaminants such as chlorinated hydrocarbons and volatile aromatics.

APPENDIX 1: LIST OF TABLES

APPENDIX 2: LIST OF FIGURES

APPENDIX 3: BIBLIOGRAPHY

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APPENDIX 4: PLANT LISTS

Table 13: Organic contaminants plant list

Latin	English	Contaminant	Indigenous to	Root depth	Succession stage	Plant density
Acer platanoides	Norway maple	BTEX	Europe	Fairly deep	Woodland	
Alnus glutinosa	Black alder	Engine oil	Europe and North Africa	Fairly deep	Woodland	
Avena sativa	Oat	TPH	Europe	Up to 50 cm	Grassland	
Betula pendula	Silver birch	PAH TCE	Europe	Fairly shallow	Woodland	
Brassica juncea	Indian mustard	PAH	Asia	90 to 120 cm	Pioneer	
Brassica napus	Rapeseed	chlorpyrifos	Europe		Pioneer	
Bromus inermis	Smooth brome	TPH	Europe and Asia	?	Grassland	
Chrysanthemum leucanthemum	Ox-eye daisy	PCB	Europe		Grassland	

Latin	English	Contaminant	Indigenous to	Root depth	Succession stage	Plant density
Cucurbita pepo	Field pumpkin	DDE DDT Chlordane PCDD PCDF				
Dactylis glomerata	Cocksfoot	TPH PAH TNT	Europe	?	Grassland	7 to 9 per m^2
Daucus carota	Wild carrot	PCB	N America		Grassland	
Elytrigia repens	Couch grass	TPH	Europe Asia	15 to 20 cm	Grassland	
Festuca spp.	Festuca	TPH PAH BTEX	Worldwide		Grassland	

Latin	**English**	**Contaminant**	**Indigenous to**	**Root depth**	**Succession stage**	**Plant density**
Festuca arundinacea	Tall fescue	Anthracene Ethylene glycol Fluoranthene Phenanthrene Pyrene TPH PAH TNT PCB	Europe	?	Grassland	6 to 12 per m²
Festuca pratensis	Meadow fescue	TPH	Europe	?	Grassland	6 to 12 per m²
Festuca rubra	Red fescue	TPH PAH	Europe Noord America	?	Grassland	6 to 12 per m²
Helianthus annuus	Common sunflower	PAH TNT	America	Up to 50 cm	Pioneer	3 to 4 per m²
Iris pseudacorus	Yellow iris	Atrazine	Europe Afrika Asia		Rugged areas	6 to 9 per m²
Juncus effusus	Common rush	PAH	Worldwide	?	Rugged areas	

Latin	English	Contaminant	Indigenous to	Root depth	Succession stage	Plant density
Lemna minor	Common duckweed	Demeton-S-methyl Malathion Metolachlor Copper sulphate Dimethomorph Flazasulfron Isoproturon Glyphosate	Worldwide			
Linum usitatissimum	Flax	TPH 2,4-D	Europe Asia	1 m	Rugged areas	
Lolium perenne	English ryegrass	TPH PAH BTEX Pentachlorophenol	Europe Asia	25 cm	Grassland	
Lotus corniculatus	Birdsfoot trefoil	TPH PAH	Europe	Up to 1 m	Grassland	

Latin	English	Contaminant	Indigenous to	Root depth	Succession stage	Plant density
Medicago sativa	Alfalfa	MTBE Ethylene glycol PAH TPH Benzene DDE	Europe	Up to 4.5 m	Rugged areas	
Melilotus officinalis	Yellow sweet clover	TPH PAH	Europe Asia	Up to 1 m	Grassland	
Miscanthus giganteus	Elephant grass	PAH	Japan	Up to 25 cm	Rugged areas	
Phaseolus vulgaris	Common bean	TNT DDE	C America		Pioneer	
Phragmites australis	Common reed	BTEX TPH MTBE Bromoform Chlorobenzene Chloroform DCE PCE TCE TNT	Europe Asia		Rugged areas	3 to 5 per m^2

Latin	**English**	**Contaminant**	**Indigenous to**	**Root depth**	**Succession stage**	**Plant density**
Pinus sylvestris	Scots pine	TPH	Europe Asia	Up to a few m	Woodland	
Plantago major	Broadleaf plantain	Imidacloprid	Europe Asia		Grassland	
Platanus occidentalis	American sycamore	TCE	America	Fairly deep	Woodland	
Poa pratensis	Blue grass	TPH PAH	Europe		Grassland	
Poaceae	Grasses	BTEX TPH PAH	Worldwide	Up to 50 cm	Grassland	
Polygonum persicaria	Lady's thumb	PCB	Eurasia		Pioneer	

Latin	English	Contaminant	Indigenous to	Root depth	Succession stage	Plant density
Populus spp.	Populars and hybrids	BTEX PAH TPH MTBE PCE TCE Pentachlorophenol Trichlorobenzene Carbon tetrachloride 1,4-Dioxane TNT Alachlor Dinoseb Atrazine Dioxane Metolachlor Metribuzin Chlorpyrifos			Woodland	
Robinia pseudoacacia	False acacia	PAH oil	N America	Fairly shallow	Woodland	
Rumex crispus	Curly dock	PCB	Eurasia		Rugged areas	

Latin	English	Contaminant	Indigenous to	Root depth	Succession stage	Plant density
Salix alba	White willow	BTEX Trifluralin Metalaxyl	Europe Asia	Fairly shallow	Woodland	
Salix babylonica	Weeping willow	MTBE TBA	China		Woodland	
Salix caprea	Goat willow	PCB	Europe Asia		Woodland	
Salix spp.	Willow	TPH BTEX PAH PCE TCE Chlorpyrifos		Fairly shallow	Woodland	
Salix viminalis	Basket willow	PAH	Europe Asia		Woodland	
Sambucus nigra	Elder	Trifluralin Metalaxyl	Europe Asia Africa		Woodland	

Latin	English	Contaminant	Indigenous to	Root depth	Succession stage	Plant density
Secale cereale	Rye	Pyrene TPH PAH	Asia	(fairly deep)	Grassland	
Solidago spp.	Goldenrods	TPH PAH PCE TCE	Europe N America S America Asia		Rugged areas	
Trifolium pratense	Red clover	TPH	Europe		Grassland	
Trifolium repens	White clover	TPH PAH BTEX PCB	Europe		Grassland	
Triticum spp.	Wheat	TPH 2,4-D	Asia		Grassland	

Latin	English	Contaminant	Indigenous to	Root depth	Succession stage	Plant density
Typha spp.	Cattail	Mineral oil Diesel Sulphonate Chloride Ethoxylate TNT Atrazine	Europe N America Asia		Rugged areas	
Zea mays	Maize	TPH Sulphonate Chloride Ethoxylate TNT Alachlor Atrazine Diazinon Temik	N America C America		Pioneer	

Table 14: Inorganic contamination plant list

Latin	English	Contaminant	Indigenous to	Root depth	Succession stage	Plant density
Arabidopsis halleri	Rockcress	Cd (hyperaccumulator) Zn (hyperaccumulator)	Europe		Rugged areas (stony substrates)	
Beta vulgaris	Beets	As Cs	Mediterranean		Pioneer	
Brassica juncea	Indian mustard	As Cu Cd Cr (VI) Ni (hyperaccumulator) Zn Se 137Cs 238U	Asia	90 to 120 cm	Pioneer	
Brassica napus	Rapeseed	Cd Cu Zn	Europe		Pioneer	
Festuca arundinacea	Tall fescue	Zn Se 137Cs	Europe	?	Grassland	6 to 12 per m^2

Latin	English	Contaminant	Indigenous to	Root depth	Succession stage	Plant density
Festuca rubra	Red fescue	134Cs	Europe North America	?	Grassland	6 to 12 per m^2
Helianthus annuus	Common sunflower	As Cd Zn Ni I U 226Ra 238U 90Sr 137Cs	America	Up to 50 cm	Pioneer	3 to 4 per m^2
Linum usitatissimum	Flax	Cd	Europe Asia	1 m	Rugged areas	
Lolium perenne	English ryegrass	134Cs 58Co	Europe Asia	25 cm	Grassland	
Medicago sativa	Alfalfa	Zn Cd Ni	Europe	Up to 4.5 m	Rugged areas	
Phaseolus vulgaris	Common bean	As	C America		Pioneer	

Latin	English	Contaminant	Indigenous to	Root depth	Succession stage	Plant density
Phragmites australis	Reed	Th U 137Cs	Europe Asia		Rugged areas	3 to 5 per m²
Populus spp.	Populars and hybrids	As Zn Cd			Woodland	
Pseudotsuga menziesii	Douglas fir	Cd	N America		Woodland	
Pteris cretica	Cretan brake	As (hyperaccumulator)	Europe Asia Africa		Rugged areas/Woodland (on rocky substrates)	
Rumex acetosa	Sorrel	Zn (hyperaccumulator) 137Cs	Europe		Grassland	
Salix spp.	Willow	Cd Zn 137Cs 90Sr		Fairly shallow	Woodland	
Salix viminalis	Basket willow	Cd Zn	Europe Asia		Woodland	

Latin	**English**	**Contaminant**	**Indigenous to**	**Root depth**	**Succession stage**	**Plant density**
Sedum alfredii	Sedum	Cd Zn	Asia			
Tagetes	Marigold	As	C America		Pioneer	
Trifolium repens	White clover	As 134Cs	Europe		Grassland	
Typha spp.	Cattail	226Ra	Europe N America Asia		Rugged areas	
Zea mays	Maize	Cd	N America C America		Pioneer	