EIP-AGRI Focus Group

Protecting agricultural soils from contamination

MINIPAPER 3: Biological remediation of contaminated agricultural soils

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1. Introduction

Agricultural practices associated with inadequate use of fertilisers (inorganic and organic materials) and pesticides or certain industrial activities can lead to soil contamination by heavy metals and metalloids, nutrient accumulation and organic pollutants (see mini-paper about sources of soil contamination).

A contamination is the presence of an undesired element or constituent affecting the quality of a material or natural environment. The different national legislations define guide values or threshold values for different organic and inorganic contaminants in the soil, mostly based on total soil concentration. If threshold values are exceeded, there is a clear obligation to remediate this site to a level either below the threshold value or according to the obligations imposed by the authorities. However, if the analysis results are only approaching but not exceeding guide or threshold values, this is a clear warning sign that the food use of the crop products should be restricted, and the potential source of the contaminants needs to be identified and eliminated.

Soil remediation methods may be applied *in situ*, *on site* and *off site*. Methods for *ex situ* / *off site* are based on excavation for treatment. The *in situ* methods leave the soil where it is (no excavation). *In situ* application of either physical, chemical or biological methods (or combinations) keep the soil structure largely intact and reduce the adverse impacts on soil life but face the difficulty to bring the remediating agents in contact with the contaminant. The *on site* methods excavate the soil for a specific treatment (biological, physical or chemical) that takes place directly at the field (no further transport). After a successful treatment, the soil is re-filled at the same place again. The *off site* methods may apply the same methods as *on site* but at a distant place where centralised technical infrastructure is available (requires extensive soil transport efforts). Methods based on physical, chemical and thermal processes (such as solidification or vitrification), degrade soil functions, drastically modify the soil physical, chemical and biological properties, and are too disruptive to be used in agricultural soils.

Bioremediation and phytoremediation are the so-called soft (or gentle) remediation practices which take advantage of soil biological processes to promote their natural remediation and are characterised by low cost, low demand for infrastructure and low carbon footprint, also they are considered environmental friendly technologies (Adriano et al., 2004; Bernal et al., 2007). These techniques help recovering the basic processes that define ecosystems functionality and sustainability. Although the bioremediation and phytoremediation techniques are well known and science-based for the scientific community, the technical sector, farmers, advisers and policy-makers are not always aware of the different options, advantages and applications of these methods. There is a general lack of knowledge about the different remediation options for specific cases; e.g. very frequently phytoremediation is only associated to phytoextraction, which is not the most efficient solution for all cases of soil pollution.

BOX 1. Biological remediation methods

Bioremediation techniques are based on the action of microorganisms for the degradation or immobilisation of the pollutants in the soil, mainly for organic contaminants.

Phytoremediation consists in the use of the plants and their associated microorganisms to remove (phytoextraction), to immobilise (phytostabilisation or phytoimmobilisation), to volatilise (phytovolatilisation) or to degrade (phytodegradation) the soil contaminants, occasionally supported by the use of organic or inorganic soil amendments for partial degradation, immobilisation or improved bioavailability of the contaminants.

The objective of the present mini-paper is to clarify the potential of the different *in situ* biological- and phytoremediation techniques for their application to different inorganic or organic soil contaminants in agricultural areas.

2. Biological remediation technologies

The total element concentration allows soil classification as contaminated or not based on the national regulations, but bioavailability of contaminants decides if safe food or feed can be produced even if soil contaminants are present. Then, the toxicity of any contaminant depends not only on its total concentration, but also on its availability in the soil. Bioavailability or potential availability of a given element in soil means that it is present in the soil solution (bioavailable) or that it may be released under changing physical or chemical conditions in the soil (potentially available); the bioavailable fraction can be absorbed by plants through the root system (Harmsen, 2007). The availability of the contaminants depends on the soil properties affecting the adsorption-desorption process, precipitation, cation exchange and other soil processes, including pH, organic matter, clay fraction and iron and manganese oxides. For example, liming acidic soils to raise the pH is the simplest and most cost-effective method to decrease metal bioavailability.

The type of contaminant, its concentration and availability in the soil define the remediation technology to be used for effective soil recovery. Soil organic and inorganic amendments, such as compost, biochar, peat, lime, gypsum, silicates, Fe-/Al-/Mn-oxides, nano-composites and other chemical reagents or combination products, have proven their efficacy in soil remediation for bio- or phytoremediation strategies (Bernal et al., 2007; Bolan et al., 2011; Hilber et al., 2017; Pardo et al., 2017a) as they affect pollutant availability. In fact, the organic amendments can activate the soil microbial population (biostimulation) and/or introduce specific microorganisms able to degrade organic pollutants (bioaugmentation), enhancing the capacity of the indigenous soil organisms to degrade organic contaminants in soils (Weil and Brady, 2017). But they can also reduce bioavailability of cationic heavy metals such as lead, zinc, or cadmium. However, the stabilisation of anionic contaminants such as arsenic requires the use of modified carbon- or clay mineral-based additives or other inorganic amendments, such as iron oxides (Arco-Lázaro, et al., 2018; Dieguez-Alonso et al., 2019).

The different phytoremediation methods take into account the mechanisms or strategies naturally developed by the plants to protect themselves from contamination: exclusion, regulation of absorption and transport to the shoot, and accumulation (Dickinson et al., 2009; Zhao and McGrath, 2009). Excluder plants maintain very low levels of toxic elements in their aerial part, due to the regulation of the absorption and transport mechanisms; they are useful for phytostabilisation or phytoimmobilisation. Accumulator and hyperaccumulator plants store toxic elements preferably in the aerial part due to an efficient transport from roots to shoots, resulting in a concentration ratio between shoot and root greater than one (Kidd et al., 2009); hyperaccumulators and accumulators of high biomass are used for effective phytoextraction, in which the plant biomass can be used for phytomining and energy production, respectively. Phytovolatilisation techniques use plants that absorb soil contaminants and turn them into volatile species; this can be used in the remediation of soils contaminated with organic compounds (e.g. chlorinated hydrocarbons) and/or elements able to form volatile species, such as selenium (Zhao and McGrath, 2009).

Box 2. Phytoremediation technologies for contaminated soils.

Phytostabilisation or phytoimmobilisation: Reduction of the availability (fixation) of the contaminants in the soil (rhizosphere) and retention in the roots.

Phytoextraction: Extraction of the toxic elements from the soil by accumulation in harvestable parts of the plants.

Phytovolatilisation: Absorption of soil contaminants, transformation into volatile species and volatilisation into the atmosphere

Phytodegradation: Biodegradation of organic toxic compounds in the rhizosphere by plant root associated microorganisms.

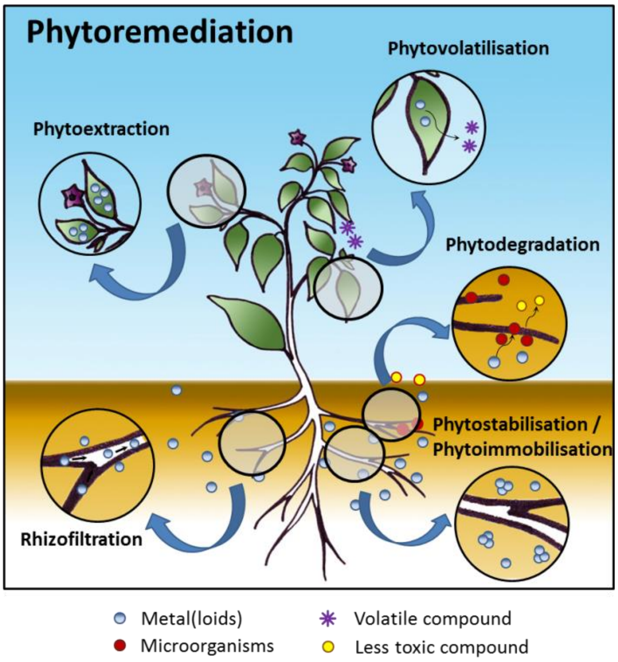

Figure 1. Phytoremediation processes for inorganic contaminants (heavy metals and metalloids) and organic contaminants (adapted from Pardo, 2013).

However, the selection of the plant species for a specific phytoremediation method should be also based on the climatic and soil conditions. In fact, plant species adapted to North-European climates (such as willow) are not suitable for Mediterranean climates. Therefore, the use of autochthonous plant species is recommended over exogenous species for effective remediation purposes.

3. Remediation of soils with inorganic contaminants

The European Environment Agency (EEA, 2016) confirms that as much as 35% of soils in Europe are contaminated with inorganic compounds. This may apply both to sites of former industrial activity and to agricultural areas located in their vicinity. The main inorganic contaminants of soils include heavy metals and metalloids (trace elements, TEs). The presence of TEs in soils in concentrations exceeding the acceptable reference limits excludes these areas from producing crops for human or animal consumption. Plants have natural mechanisms to take up metals which are essential nutrients, such as Cu, Co, Fe, Mo, Mn, Ni, and Zn, but others have no known physiological activity function, such as Cd, Pb or Hg (Lasat, 2002).

When the level of contaminants is high, it may become toxic for plants, preventing or limiting their growth and proper development, one of the solutions is phytostabilisation, using a vegetation cover composed of species with high tolerance to contamination by exclusion. Such cover may reduce by itself the migration of metals in the soil as well as to the aboveground parts of plants by stabilising them within the root zone (Figure 1). The application of mineral and/or organic amendments to the polluted soils can reduce metal mobility due to changes in soil properties - aided phytostabilisation. Organic soil additives like biochar and compost may both improve physico-chemical soil characteristics and immobilise heavy metals, thereby reducing the uptake of contaminating elements by crops and the leaching towards groundwater (Beesley et al., 2010). This beneficial effect is applicable in phytostabilisation of Cu contaminations that extensively affect vineyard soils in traditional winegrowing regions (Soja et al., 2018). For this technology, tolerant plants by exclusion are used, which may further enhance heavy metal immobilisation due to rhizosphere processes - phytoimmobilisation (Mench et al., 2010). These plants can be perennial grasses, like Miscanthus, cordgrass, switchgrass or other energy crops like Virginia mallow (Pogrzeba et al., 2017; 2018a, b; Krzyżak et al., 2017). These plant species may also provide the opportunity to gain profit from the harvested biomass, as a source for further industrial processes, e.g. production of fibres, oil, or energy (Weyens et al., 2009; Pavel et al., 2014).

Under Mediterranean climate, the problem of heavy metal toxicity is exacerbated by associated problems, such as soil salinity, and low concentration of organic matter and nutrients in the soils. Also, the plants must be adapted to the particular climatic conditions, with dry periods even during winter, and long, dry and hot summers. To cope with such problems, autochthonous plant species are generally used for phytostabilisation, such as milk thistle, false yellowhead, wild tobacco or Syrian bean-caper (Martínez-Fernández et al., 2014; Pardo et al., 2017b), as they inhibit the translocation of heavy metals and also As to harvestable parts. In this sense, halophytic native plant species (e.g. Mediterranean saltbush) have clear advantages as they usually possess high tolerance of extreme soil conditions and high TEs concentrations in the soil (Clemente et al., 2012). To cope with the poor fertility (organic matter and nutrients) of contaminated soils from semi-arid areas, efficient phytostabilisation methods are based on the application of compost or the co-culture of N_2-fixing species (such as *Bituminaria bituminosa*) with native species (Clemente et al., 2012; Arco-Lázaro et al., 2017).

In areas where heavy metal contamination does not greatly exceed the established limits (or geochemical background levels), extraction of metals by accumulator (or hyperaccumulator) plants by phytoextraction, can be a feasible option. The plants (annual or perennial species) should be tolerant (without growth reduction) to the metals by accumulation in their harvestable parts. Hyperaccumulator species are the most efficient plants for metal phytoextraction. Simple mass-balance calculations (metal concentration in the harvestable biomass × amount of harvestable biomass) show if phytoextraction is potentially feasible in a particular case. Such technology may be applicable in moderately Cd contaminated soils, or Zn/Cd contaminated soils using a particular ecotype of the hyperaccumulator *Noccaea caerulescens* (previously named *Thlaspi caerulescens*) (Chaney et al., 2007; McGrath et al., 2006). The phytoextraction of valuable metals (such as Ni) is called phytomining, as the valuable metal is concentrated in the harvested biomass with respect to the original soil. The metal can be easily recovered, either from the dry biomass or from the ashes, after biomass incineration. Although hyperaccumulator species can accumulate extremely high concentration of heavy metals, they generally have low biomass. Then, high biomass accumulator species (but not hyperaccumulator) can also be effective for phytoextraction, such as willow or poplar, or even annual crops (e.g. tobacco and sunflower; Thijs et al., 2018). However, phytoextraction is only a feasible option in low or moderately contaminated soils.

The induced phytoextraction is an option to improve the effectiveness of the extraction by accumulator (non-hyperaccumulator) crops. In such technique, the hyperaccumulation is induced in an accumulator species of high biomass by adding metal-solubilising agents to the soil, such as synthetic chelators (EDTA, HEDTA, DTPA, EDDS, NTA etc.) or natural low molecular weight organic acids. Plants used for induced phytoextraction are high biomass species: annual crops such as maize, tobacco, Indian mustard, oat, barley, pea and sunflower (Evangelou et al., 2007) or perennial species, such as poplar or miscanthus. However, the induced phytoextraction has been used only in small scale experiments due to the high costs of amendments, ecotoxicological concerns and potentially increased toxicity caused by the high concentration of soluble metals remaining in the soil after crop harvesting. Some soil additives, like biochar or clay minerals that immobilise the residual metals after plant harvest may counteract the adverse effects of excessive mobilisation. When the amount of contaminating element in the soil is large and the area-based annual removal rates by accumulator plants are low, it may take too many years to evidence for a significant remediation effect by phytoextraction. In such cases, the use of phytostabilisation technology is recommended.

In situations where both inorganic and organic contaminants are present in the soil, the remediation programme can combine phytoremediation (or aided phytoremediation with organic or inorganic additives) with microbial remediation: bioaugmentation (microbes) or biostimulation (Agnello et al., 2016). Microorganisms can interact with inorganic contaminants via many mechanisms: biosorption (metal sorption to cell surface by physico-chemical mechanisms), bioleaching (heavy metal mobilisation through the excretion of organic acids or methylation reactions), biomineralisation (heavy metal immobilisation through the formation of insoluble sulphides or polymeric complexes), intracellular accumulation, and enzyme-catalysed transformation (redox reactions) (Lloyd et al., 2005). For example, microbes like *Bacillus*, *Enterobacter*, *Escherichia*, *Pseudomonas* help in bioremediation of metal-contaminated soil (Kotas and Stasicka, 2000). Bioaugmentation-assisted phytoextraction optimises the synergistic effect of plants and microorganisms for the cleaning-up of soils contaminated by heavy metals (Huguenot et al., 2015). In many cases the combined use of plants, microorganisms and stabilising amendments like biochar, compost or clay minerals is the most advantageous option for the treatment of soil contaminated with inorganics and organics and can generally lead to a decrease of plant metal concentration and translocation (Agnello et al., 2016).

Table 1. Effectiveness of amendments in heavy metal and metalloid immobilisation for phytostabilisation.

Amendment	Metal(loid) immobilisation						
	Pb	Cd	Zn	Cu	Ni	Cr	As
Phosphorus compounds	++	+	+	+	+/-	+	-
Organic matter	+/-	+/-	+/-	+/-	+/-	+/-	+/-
Biochar (*modified biochar)	+	+	+	+/-	+	+*	+*
Clay minerals	+	+	++	+	+	+	+
Alkaline materials	+/-	+	++	+	+	+/-	-
Fe oxides	+/-	+/-	+/-	+/-	+/-	+/-	++
Mn and Al oxides	+	+	+	+	+/-	-	+

(++): very good; (+): good; (+/-): inconclusive; (-): negative.

Inorganic contaminants include also non-metallic elements called metalloids, with some properties of heavy metals, but with different behaviour from those of metals, including arsenic (As), antimony (Sb) or selenium (Se). Phytoremediation of metalloids has mainly been developed for As contaminated soils, due to the As-hyperaccumulator character of the fern *Pteris vittata* (Su et al., 2008). In such species As is translocated from roots to fronds extremely efficiently and mainly in the form of arsenite. In non-hyperaccumulators arsenate is retained in the roots. Phytostabilisation of As contaminated soils with the incorporation of amendments with high adsorption capacity has been proposed as an efficient approach for As soil remediation through its *in situ* fixation. Iron-rich materials (iron oxides or Fe-rich industrial by-products, such as lamination slag and red mud), have been considered as suitable soil amendments because of their potential for As immobilisation in soils, reducing As bioavailability and avoiding its accumulation in crops and transfer to the food chain (Arco-Lázaro et al., 2018; Madeira et al., 2012).

The problem of Se deficiency in humans and animal is more frequent than Se toxicity. Its deficiency in food and forage plants can occur both as a consequence of low Se concentration in the soil and low bioavailability for uptake of soil Se in plant roots and mycorrhiza. For toxicity problems to occur, a combination of high soil Se concentration and high bioavailability for Se uptake in the roots is needed. Selenium accumulation in plants varies in their ability to transform inorganic Se to organic forms. Eventually, Se can be methylated and volatilised by plants. Then, excessive concentration of Se in contaminated soils can be taken up and volatilised by plants and associated microorganisms

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(Pilon-Smith, 2005) through phytovolatilisation technology. Soils rich in Se can be used for cultivation of Se-rich food crops (e.g. wheat) that can be used for Se fortification of similar crops from Se-deficient areas. Also, the cultivation of Se accumulator plants can be considered as a biofortification method. Therefore, biofortification is recommended as a bioremediation option in high Se soils (Zhao and McGrath, 2009).

4. Remediation of soils with organic contaminants

Organic contaminants consist on carbon-based molecules. A multitude of different chemicals may act as soil contaminants, such as polycyclic aromatic hydrocarbons (PAH), dioxins (PCDD/F), polychlorinated biphenyls (PCB), pesticides, halogenated compounds (e.g. chlorinated hydrocarbons, fluorinated alkyl compounds), explosives, phthalates (as an example for compounds acting as endocrine disruptors), drugs (e.g. antibiotics, veterinary drugs), veterinary and medical or personal care products, microplastics, etc. Sources of organic contaminants in agricultural soils are mainly associated to pesticides, sewage sludge or low-quality compost (see mini-paper on sources of pollution).

Theoretically, the organic contaminants can be degraded up to 100 % of the original amount to harmless compounds – contrary to the situation with toxic inorganic elements. However, the complete degradability can only occur to certain contaminants that are easily accessible for microorganisms or for chemical modification. Therefore, a significant fraction of contaminants, unfortunately including very toxic and persistent ones (e.g. the PAH benzo(a)pyrene), are so difficult to attack even for the most inventive microorganisms that the pollutants may persist for decades in the soil. In such cases, only the use of drastic methods may help, accompanied by serious disruption of the integrity of soil and soil life.

The technologies for the remediation of a soil contaminated by organic pollutants may be applied *in situ*, *on site* and *off site*. The natural attenuation is the favourite method for all who aim for minimal interference. This method relies on the natural degradation by the *in situ* existing soil microorganisms and on the naturally occurring chemical and physical processes in soil. This method works with minimum technical effort, but it may take many years for satisfying remediation efficiency, depending on the biological degradability of the contaminant. By enhanced natural attenuation, the activity of the microorganisms is supported or enhanced by different soil amendments.

Therefore, **bioremediation** is an active microorganisms-mediated technology, which uses native or inoculated microorganisms to degrade organic contaminants in soil, groundwater, sludge and solids. The microorganisms, mainly bacteria and fungi, break down contaminants by using them as an energy source or metabolising them with an energy source (Vidali, 2001; Cycoń et al., 2017; Morillo and Villaverde, 2017; USEPA, 2019). There are different soil bioremediation methods available to be applied *in situ*: **biostimulation** (enhanced bioremediation), **bioaugmentation** and **bioventing** (Vidali, 2001).

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Box 3. Bioremediation of organic contaminants.

Biostimulation: Enhancement of autochthonous microbial population and activity by nutrients, oxygen, or other amendments to promote the degradation of organic contaminants.

Bioaugmentation: The addition of microorganisms (autochthonous, allochthones, or even genetically modified) specially selected to decompose organic contaminants.

Bioventing: Stimulation of the indigenous bacteria by supplying air and nutrients through wells to the soil.

Biostimulation uses indigenous (naturally occurring) microorganisms to degrade (metabolise) organic contaminants found in soil, converting them to innocuous end-products. Nutrients, oxygen, or other amendments may be used to enhance bioremediation and contaminant desorption from subsurface materials, once degradation is affected by different conditions (e.g. soil moisture, soil pH, oxygen content, nutrient content, temperature, type of soils, and the presence of other contaminants; Vidali, 2001). It is as a long-term technology, which may take several years for complete soil clean-up (Gavrilescu, 2009). **Bioaugmentation** is recommended for sites where the number of autochthonous microorganisms is not sufficient to degrade the contaminant load present, or when the native consortia do not have the catabolic pathways necessary to metabolise those molecules (Cycoń et al., 2017). It involves the addition of microorganisms either autochthonous, allochthones, or even genetically modified, to the contaminated sites (Cycoń et al., 2017). In the first method, microorganisms are isolated from the contaminated environment, and reinjected onto the same site as enriched cultures, or as a consortium. For allochthones bioaugmentation, microorganisms are collected from another site, and added to the contaminated soil, also as enriched cultures or as a consortium. Gene bioaugmentation involves the use of GM microorganisms, with genes encoding the enzymes responsible for the degradation of the specific contaminants (Cycoń et al., 2017). The use of compost as soil amendment is also a kind of bioaugmentation (due to the large variety of different microorganisms present in compost), but also serves biostimulation (due to the organic matter and the nutrients that it provides). **Bioventing** involves supplying air and nutrients through wells to contaminated soil to stimulate the indigenous bacteria. Bioventing employs low air flow rates and provides only the amount of oxygen necessary for the biodegradation, while minimising volatilisation and release of contaminants to the atmosphere (Vidali, 2001).

Plants are the most eminent supporters of microbial life in soil. Their roots excrete assimilates coming down from the photosynthesising leaves and nourishing soil microbes. For this reason, they accumulate in a zone of a few millimetres around the fine roots of plants, the so-called rhizosphere. As far as soil and climatic conditions allow plant growth, the phytoremediation strategy usually excels in the contaminant degradation efficiency. But, in the case of organic contaminants, the microbial consortium is responsible for the degradation of the toxic compounds, even in phytoremediation with rhizospheric microorganisms as the main actors. The phytovolatilisation is an exception, where the plants are directly responsible for the cleaning. In this case, easily volatile pollutants may be taken up by the plant roots, transported to the leaves together with the transpiration water and finally dissipate into the atmosphere. Unfortunately, only for few contaminants, e.g. some chlorinated hydrocarbons, this may be a feasible decontamination strategy. The toxicity of the contaminants to the plants may inhibit the exploitation of the full potential.

Also, organic or inorganic sorbents may assist soil microbes by supplying a habitat to live, nutrients, or a support to be attached in an immobilised form. The most efficient sorbents for such purposes are biochar, activated carbon and zeolites. Although there have been reservations about the use of sorbents in parallel with degrading microorganisms because it was feared that a reduced bioavailability of sorbed contaminants might also reduce the degradation efficiency of the bacteria, the contrary has been observed. Apparently, an elevated abundance of the microorganisms at attractive sorbent surfaces supports the degradation of these contaminants in spite of their sorption.

Table 2. Overview of *in situ* and *on site* remediation methods for organic soil contaminants. Costs are rough estimates that may vary depending on the specific contaminant situation. For symbol explanation see footnotes.

Technology	Contaminant	Effectiveness[2]	Time needed	Ecological side-effects	Costs
Natural attenuation	[1]	from - - to +	- -	+ +	Very low
Biostimulation, bioventing, bioaugmentation	[1]	from - - to + +	-	+ +	Low to moderate
Phytoremediation, phytovolatilisation	[1]	from - - to + +	-	+ +	Low to moderate
Immobilisation	[1]	from - - to + +	+	+	Moderate
In-situ chemical oxidation or reduction	[1]	from +/- to + + [2]	+ +	+/-	Moderate to high
Thermal (steam stripping)	[1]	from +/- to + +	+ +	+/-	Moderate to high
Biological/non-biological combination methods	[1]	from +/- to + +	+	+/-	Moderate to high
Nanotechnological combination methods	[1]	from +/- to + + [2]	+	+/-	High

[1]Suitability of method needs to be tested for any specific contaminant prior to field application; [2]Depending on specific contaminant; -: low, unfavourable, long duration; +: high, beneficial, short duration.

As previously indicated, the co-occurrence of organic contaminants and metal/ metalloid contaminants are frequent in earlier industrial sites. Usually these two groups of contaminants require very different remediation strategies. However, the combination of sorption materials and biological remediation has the potential to cope with both problems. A reasonable degradation efficiency of the organic contaminants frequently is inhibited by the toxic effects of the metals. However, if the bioavailability of the toxic elements is reduced by sorption to sorbents like biochar, the biological degraders of the organic fractions can be active because the adverse effects of the toxic metals on their vitality has been reduced. Eventually, the degradation efficiency of the organic contaminant is improved and the risk of metal transfer is reduced. New materials, such as nano-zero-valent iron (nZVI) have demonstrated its efficiency for remediation of e.g. chlorinated hydrocarbons. Combination of new materials (nZVI, graphite composites with Fe-oxides, with biochar, hydrochar or activated carbon, modified clay materials with organic coatings) are seen as promising for biodegradation by immobilised enzyme systems.

If a contaminant is too recalcitrant for biological degradation, the application of sorption materials may still be helpful as a pre-treatment. The sorption capacity of biochar and related soil amendments immobilises a contaminant, and both the risk of contaminant migration towards groundwater and the undesired uptake into plants are reduced. Very persistent and hardly degradable contaminants like fluorinated alkyl compounds are candidates for such treatments if *in situ* is the preferred strategy. Other chemical treatments (such as chemical oxidation and chemical reduction) are also frequently combined with biological degradation methods. Chemical oxidation is applied for contaminants that can be oxidised, e.g. many hydrocarbons; whereas chemical reduction is applied for chlorinated compounds that need to be dechlorinated reductively (like trichloroethylene). These treatments may be followed by a biological remediation steps, for a subsequent microbiological degradation.

Pesticides constitute a particular group of organic contaminants. They are a wide group of compounds, which include insecticides (44%), herbicides (30%), fungicides (21%), and others (12%) (World consumption patterns of pesticides; Aktar et al., 2009). Although the use of pesticides permits the currently high level in agricultural productivity, the excessive or improper use of pesticide may cause major environmental and ecotoxicity problems (Aktar et al., 2009). Overuse of pesticides contaminates different environmental compartments, air, soil, surface and ground water, affecting non-target vegetation and other organisms, food commodities and human health (Aktar et al., 2009).

The majority of the plant protection products applied in agricultural fields are organic compounds, and the remediation methods for soils contaminated with pesticides could be any of those previously described for organic contaminants, including persistent organic pollutants (POP, Stockholm Convention[1]) (Caliman et al., 2011; Morillo and Villaverde, 2017). However, some of these technologies are very harsh, and more adequate to remediate industrially contaminated soils (Morillo and Villaverde, 2017; USEPA, 2019), with a heavy load of contamination. When the soil is intended for agricultural use, the selected techniques should be gentle with low disturbance of soil life and soil functionality (Morillo and Villaverde, 2017).

To reduce the concentration, eliminate or stabilise a pesticide in soil, the selected remediation technology based on physical, chemical or biological processes, could be applied *in situ*, *ex situ/on site*, or *ex situ /off site* (e.g., landfarming, composting, biopiles, bioreactors) (Vidali, 2001; Morillo and Villaverde, 2017). Biostimulation, bioaugmentation and bioventing methods are available for of soils contaminated with pesticides (Cycoń et al., 2017; Jariyal et al., 2018; Odukkathil and Vasudevan, 2016; Vidali, 2001). Morillo and Villaverde (2017) reviewed different bioremediation

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processes/technologies for pesticides contaminated soils (*in situ* and *ex situ*), indicating the specific remediation technology, the microorganisms involved, and the results in the pesticide removal. Promising results included the biodegradation by bioaugmentation of >85% of fungicides (myclobutanil, tetraconazole and flusilazole) in 20 days, in vineyard plots, using *Bacillus* strains; the biodegradation, using biostimulation and a bioavailability enhancer, of 74.3% organochlorine pesticides in 180 days in paddy field soils; and the biodegradation of up to 97% of pentachlorophenol (herbicide) by biostimulation of the endogenous flora, only in 6 days, just to name some. The use of bioaugmentation was reviewed by Cycoń et al. (2017) as the most promising technology for pesticides contaminated soils. For each specific pesticide, the microorganisms involved, the optimal conditions, and the results in the pesticide removal are included, with examples for triazine, [1]organophosphorus, organochlorinated, and pyrethroids, among other pesticides.

However, limitations of the bioremediation for soils contaminated with pesticides have been found: the microorganisms, which revealed able of degrading pesticides under laboratory conditions, are less efficient in field conditions due to the pesticide low availability (depending on their solubility and adsorption to soil organic matter and clay particles solubility) and to toxicity of other pesticides (Odukkathil and Vasudevan, 2016). For microbial growth and activity, soil pH, temperature, moisture content, available oxygen, and physical structure are of paramount importance (Vidali, 2001). Although microorganisms have been isolated in extreme conditions, most of them grow optimally under specific soil conditions, so that it is important to achieve such optimal conditions, by controlling the abovementioned variables.

More drastic methods can be applied for difficult organic contaminants, which cannot be successfully destroyed even by oxidation or reduction. In such cases only drastic *off site* thermal methods such as combustion or pyrolysis can be used, if trying to avoid dumping and sealing due to economic and environmental reasons. Those strategies are in contradiction to the principles of soil protection and circular economy. Through these thermal methods, the undesired organic contaminants disappear but also the soil organic matter, a basic component of the soil. Thus, gentle remediation techniques that avoid soil excavation and off-site treatment are frequently recommended.

[1] List of the persistent organic pollutants (POP), Stockholm Convention, Pesticide POPs (2019 list): DDT, aldrin, alpha hexachlorocyclohexane (Alpha-HCH), beta hexachlorocyclohexane (Beta-HCH), chlordane, chlordecone, dicofol, dieldrin, endrin, heptachlor, lindane, mirex, pentachlorophenol and its salts and esters (PCP), technical endosulfan and its related isomers, and toxaphene. available at: http://www.pops.int/TheConvention/ThePOPs/AllPOPs/tabid/2509/Default.aspx.

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4.1 Case studies of soil biological remediation

Case 1. Energy crops grown in arable soils contaminated with heavy metals

Site 1: agricultural land is located in the Upper Silesian Metropolitan Association, in the vicinity of a former lead and zinc works, which had been operating for over 100 years. In the last 30 years, the area had been used for the cultivation of cereal crops, especially wheat. The soil was classified as silty loam of a neutral pH and organic matter content up to 5%. The total content of Pb and Cd exceeded 4 to 6 times the limits for agricultural soils, while those of Zn exceeded for 4 to 7 times (Journal of Laws of 2016, item 1395). The bioavailable forms of Cd and Zn were approx. 5% and 2.5% of the total content, and that of Pb was below the limit of quantification.

Site 2: located in Germany in the vicinity of Leipzig, in a place where 650 000 tonnes of municipal sewage sludge were deposited (1952-1990). The soil was sandy loam, with a neutral pH and extremely high content of organic matter (approx. 33%). The total content of Pb, Cd and Zn in soil was similar to the level found in the site 1, with very low bioavailability of metals.

Energy crops – *Miscanthus x giganteus*, *Sida hermaphrodita*, *Spartina pectinata* and *Panicum virgatum* - were grown in both sites during three seasons. Phytoextraction of heavy metals was very limited in site 2 due to their low bioavailability linked to the high organic matter content. *Miscanthus x giganteus* was able to extract the highest amount of Cd and Zn among all the tested species. The species *S. pectinata* was revealed useful for phytostabilisation, with low metal uptake in the aboveground biomass; *S. hermaphrodita* accumulated Cd and Zn while *P. virgatum* had high concentration of Zn in shoots with low concentration of Pb and Cd.

Case 2. Miscanthus biomass options for contaminated and marginal land: quality, quantity and soil interactions

Several novel *Miscanthus* seed-based hybrids were tested on marginal and contaminated lands at three locations in Europe (Poland, Germany, United Kingdom). *Miscanthus* is an alternative non-food crop, which biomass is used for energy (combustion or biogas by anaerobic digestion). The slightly increased heavy metal concentrations in the biomass cultivated at heavy metal contaminated soil had no negative effects on the ash melting behavior. Harvesting before winter seems to be favorable for anaerobic digestion, with a slightly higher substrate specific methane yields. Increase heavy metal content in biomass did not affect negatively the anaerobic digestion. *Miscanthus* was confirmed as a safe and profitable crop for marginal and contaminated soils.

Figure 2. Miscanthus in a soil contaminated with heavy metals.

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Case 3. Restoration program of an agricultural soil polluted by a mining spill

The agricultural land was affected by a toxic pyritic spill of acid water and sludge in 1998. After the removal of the sludge and the first top layer of the affected soil by heavy machinery, the soil (loam texture, carbonates<0.5 %, pH range 2-7) remained contaminated by Zn, Pb, As, Cd and Cu, with a range of concentrations and solubility. The remediation program was: 1. Active phytoremediation (2 years) with two successive crops of *Brassica juncea* and the addition of organic amendments (compost and cow manure) and lime (in the acid patches) for extraction of the soluble metal fraction; 2. Natural attenuation without external intervention (5 years) and the colonisation with wild species; and 3. The restoration of the site (5 years) by re-vegetation with selected native shrubs (*Retama sphaerocarpa*, *Tamarix gallica*, *Rosmarinus officinalis* and *Myrtus communis*), for the re-establishment of the soil ecological services with a permanent vegetation (Clemente et al., 2006; de la Fuente et al., 2014).

Figure 3. Phytoremediation program: left soil before and right soil after restoration.

Case 4: Innovative case studies for remediation of organic soil contaminants.

A combination of indigenous willows and fungi decreased petroleum hydrocarbons in contaminated soils by 65-75 %, even under cold climates (Robichaud et al., 2019).

A soil co-contaminated by heavy metals and organic pesticides was remediated by a combination method of phytoremediation with alfalfa and biochar to decrease the toxicity (Zhang et al., 2019).

The development of nanoscale zero valent iron materials was developed for combination applications with biological, physical and chemical remediation methods (Jiang et al., 2019).

Case 5. Bioremediation of soils with pesticides

Bioaugmentation: Use of a biosurfactant-producing bacterial consortium (*Bordetella petrii I* GV 34, *B. petrii* II GV 36 and *Achromobacter xyloxidans* GV 37) to degrade endosulfan (ubiquitous organochlorine insecticide, POP), and its metabolites in a contaminated surface and subsurface agricultural soil. The complete removal of alpha and beta endosulfan was observed in 25 days in a simulated soil profile reactor (Odukkathil and Vasudevan, 2016).

Bioaugmentation: Use of a microbial consortia (*Brevibacterium frigoritolerans, Bacillus aerophilus, and Pseudomonas fulva*), isolated from aged contaminated soil with phorate (organophosphorous insecticide, banned in EU and used with limits in the US). In a sandy loam soil the highest phorate removal was between 97.65 and 98.31%, reached in 42 days (Jariyal et al., 2018).

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5. Conclusions

Agricultural soils may suffer from a large diversity of inorganic or organic contaminants, which may reduce the quality of food crops by increased pollutant concentrations or endanger groundwater or surface waters. Research in environmental engineering methods has resulted in a large toolbox of biological, physical or chemical methods to mitigate the contaminant impacts and to remediate the contaminated soils. Under favourable conditions, a deliberate use of such methods may make soils fit again for agricultural crop production. Depending on the specific pollutant situation, soil and climate conditions, the most appropriate method using plants, microorganisms, organic or inorganic soil amendments individually or in combination should be selected. The vast field of potential contaminant situations and emerging pollutants requires the continued development of new remediation strategies, including combinations of already tested and proven options as well as the development of innovative deployment techniques.

Although several biological methods based on plants and microorganisms and their interactions have been developed for soil remediation, the practical application of some of them needs to be tested under real conditions. Not a single method can be applied to all soil, climatic and pollution situation, but there is an optimum method for each particular case. The inorganic contaminants can be removed, stabilised, immobilised, and in some cases volatilised by specific plant species, under the support of soil amendments to modify the availability of the contaminants. Biological degradation of organic contaminants can be carried out by specific microorganisms, including those present in the soil rhizosphere, which can be enhanced or specifically inoculated for efficient bioremediation. Novel processes based on combination of physical, chemical and biological methods are under development (such as chemical oxidation and reduction combined with biological degradation methods for organic pollutants). The combination of biological methods and new materials (such as new bio-based materials, nano-materials of zero-valent iron and combinations with biochar or other soil amendments) seems to be the future trend for innovative decontamination solutions. Considering the complexity of organic contaminants in the soil, and the multitude of environmental factors affecting a successful degradation, a rapid increase of the biological and non-biological toolbox to deal with contaminated soils in-situ is highly desirable.

The final use of the soil should be considered in advance, for selecting the most appropriate remediation method. At this point the agricultural, environmental, societal and economic implications of non-action versus environmentally friendly remediation should be considered. Nowadays, the production of non-food crops, such as energy, ornamental or fibre crops is seen as an agricultural alternative for remediated agricultural areas. The establishment of a healthy and self-sustaining vegetation cover should support the provision of ecosystem services.

6. Research needs

- Develop strategies for new pollutants: microplastics, fluorinated compounds, endocrine disruptors, drug residues.
- Strategies for mixed combined contamination: organic and inorganic pollutants, or heavy metals and metalloids.
- Define autochthonous plant species for different climatic conditions and specific for each remediation method.
- Long-term experiments for validation of the remediation techniques: efficiency versus time and cost.

- Establishment of criteria for remediated soils: pollutant bioavailability, risk assessment, soil health and biodiversity.
- Define the final use of the soil to ensure business viability for the farmers.
- Assessment of the social costs of delaying remediation of brownfields or in urban areas.

7. Ideas for innovations

The development of new soil amendments to retain organic and inorganic contaminants to fix them in the soil to reduce their bioavailability, toxicity and leaching risk.

The combination of different remediation methods and materials is a continuing trend that surely will provide more new technologies to further increase the remediation success.

Evaluation of the agricultural, environmental, societal and economic implications of the different remediation methods is necessary for selecting the adequate technology.

The production of non-food crops, such as energy, ornamental or fibre crops is seen as an agricultural alternative for remediated agricultural areas.

The establishment of a healthy and self-sustaining vegetation cover should support the provision of ecosystem services.

8. Proposal for potential operational groups

1. Alternative plant crops for low quality agricultural or marginal land: advantages, disadvantages, and benefits for farmers and for the environment.
2. Linking the crop, and soil amendments with the soil health status.
3. Non-food crops as alternatives for soil remediation in Mediterranean climates.
4. Evaluation of bioavailability, bioaccesibility and solubility of contaminants in agricultural soils to protect crops and water.
5. Carbon sequestration in agricultural soils to mitigate climate change and soil contamination.
6. Buffer strips near surface waters as biodiversity areas and contaminant sinks.

The potential operational groups 2 and 4 can be relevant to define the main soil contaminants of agricultural origin and establish preventative measures (see mini-paper on Agricultural sources of soil contamination) in addition to developing biological remediation practices.

> Further research needs coming from practice, ideas for EIP AGRI operational groups and other proposals for innovation can be found at the final report of the focus group, available at the FG **webpage**.

9. References

Adriano, D.C., Wenzel, W.W., Vangronsveld, J., Bolan, N.S., 2004. Role of assisted natural remediation in environmental cleanup. Geoderma 122, 121–142.

Agnello, A.C., Bagard, M., Van Hullebusch, E.D., Esposito, G., Huguenot, D., 2016. Comparative bioremediation of heavy metals and petroleum hydrocarbons co-contaminated soil by natural attenuation, phytoremediation, bioaugmentation and bioaugmentation-assisted phytoremediation. Science of the Total Environment, 563, 693–703.

Aktar, W., Sengupta, D., Chowdhury, A., 2009. Impact of pesticides use in agriculture: their benefits and hazards. Interdisciplinary Toxicology, 2(1), 1–12.

Arco-Lázaro, E., Martínez-Fernández, D., Bernal, M.P., Clemente, R., 2017. Response of *Piptatherum miliaceum* to co-culture with a legume species for the phytostabilisation of trace elements contaminated soils. Journal of Soils and Sediments 17, 1349–1357.

Arco-Lázaro, E., Pardo, T., Clemente, R., Bernal, M.P., 2018. Arsenic adsorption and plant availability in an agricultural soil irrigated with As-rich water: Effects of Fe-rich amendments and organic and inorganic fertilisers. Journal of Environmental Management 209, 262-272.

Beesley, L., Moreno-Jiménez, E., Gomez-Eyles, J.L., 2010. Effects of biochar and greenwaste compost amendments on mobility, bioavailability and toxicity of inorganic and organic contaminants in a multi-element polluted soil. Environmental Pollution 158 (6), 2282-2287.

Bernal, M.P., Clemente, R., Walker, D.J., 2007. The role of organic amendments in the bioremediation of heavy metal-polluted soils. In: Gore, R.W. (Ed.), Environmental Research at the Leading Edge. Nova Science Publishers Inc, New York, pp. 1–57.

Bolan, S.N., Park, J.H., Robinson, B., Naidu, R., Huh, K.Y., 2011. Phytostabilization: a green approach to contaminant containment. Advances in Agronomy 112, 145–204.

Caliman, F.A., Robu, B.M., Smaranda, C., Pavel, V.L., Gavrilescu, M., 2011. Soil and groundwater cleanup: benefits and limits of emerging technologies. Clean Technology and Environmental Policy 13, 241–268.

Chaney, R.L., Angle, J.S., Broadhurst, C.L., Peters, C.A., Tappero, R.V., Sparks, D.L., 2007. Improved understanding of hyperaccumulation yields commercial phytoextraction and phytomining technologies. Journal of Environmental Quality 36,1429-1443.

Clemente, R., Almela, C., Bernal, M.P., 2006. A remediation strategy based on active phytoremediation followed by natural attenuation in a soil contaminated by pyrite waste. Environmental Pollution 143, 397–406.

Clemente, R., Walker, D.J., Pardo, T., Martínez-Fernández, D., Bernal, M.P., 2012. The use of a halophytic plant species and organic amendments for the remediation of a trace elements-contaminated soil under semi-arid conditions. Journal of Hazardous Materials 223-224, 63-71.

Cycoń, M., Mrozik, A., Piotrowska-Seget, Z., 2017. Bioaugmentation as a strategy for the remediation of pesticide-polluted soil: A review. Chemosphere 172, 52–71.

de la Fuente, C., Pardo, T., Alburquerque, J.A., Martínez-Alcalá, I., Bernal, M.P., Clemente, R., 2014. Assessment of native shrubs for stabilisation of a trace elements-polluted soil as the final phase of a restoration process. Agricultural Ecosystems and Environment 196, 103-111.

Dickinson, N.M., Baker, A.J.M., Doronila, A., Laidlaw, S., Reeves, R.D., 2009. Phytoremediation of inorganics: realism and synergies. International Journal of Phytoremediation 11, 97–114.

Dieguez-Alonso, A., Anca-Couce, A., Frišták, V., Moreno-Jiménez, E., Bacher M., Bucheli, T.D., Cimò, G., Conte, P., Hagemann, N., Haller, A., Hilber, I., Husson, O., Kammann, C.I., Kienzl, N., Leifeld, J., Rosenau, T., Soja, G., Schmidt, H.P., 2019. Designing biochar properties through the blending of biomass feedstock with metals: Impact on oxyanions adsorption behavior. Chemosphere 214, 743-753.

EEA, 2016. Report 07/2016. Soil resource efficiency in urbanised areas. Analytical framework and implications for governance, European Environment Agency. pp. 90.

Evangelou, M.W., Ebel, M., Schaeffer, A., 2007. Chelate assisted phytoextraction of heavy metals from soil. Effect, mechanism, toxicity, and fate of chelating agents. Chemosphere 68(6), 989-1003.

Gavrilescu, M., 2009. Emerging processes for soil and groundwater cleanup - potential benefits and risks. Environmental Engineering and Management Journal 8(5), 1293–1307

Harmsen, J., 2007. Measuring bioavailability: from a scientific approach to standard methods. Journal of Environmental Quality 36(5), 1420-1428.

Hilber, I., Bastos, A.C., Loureiro, S., Soja, G., Marsz, A., Cornelissen, G. Bucheli, T.D., 2017. The different faces of biochar: contamination risk versus remediation tool. Journal of Environmental Engineering and Landscape Management 25, 86-104.

Huguenot, D., Bois, P., Cornu, J.Y., Jezequel, K., Lollier, M., Lebeau, T., 2015. Remediation of sediment and water contaminated by copper in small-scaled constructed wetlands: effect of bioaugmentation and phytoextraction. Environmental Science and Pollution Research, 22, 721–732.

Jariyal, M., Jindal, V., Mandal, K., Gupta, V.K. Singh, B., 2018. Bioremediation of organophosphorus pesticide phorate in soil by microbial consortia. Ecotoxicology and Environmental Safety 159, 310–316.

Jiang, D., Zen, G., Huang, D., Chen, M., Huang, C., Wan, J., 2019. Remediation of contaminated soils by enhanced nanoscale zero valent iron. Environmental Research 163, 217-227.
Kidd, P., Barceló, J., Bernal, M.P., Navari-Izzo, F., Poschenrieder, C., Shilev, S., Clemente, R., Monterroso, C., 2009. Trace element behaviour at the root–soil interface: Implications in phytoremediation. Environmental and Experimental Botany 67, 243–259.
Kotas J., Stasicka Z., 2000. Chromium occurrence in the environment and methods of its speciation. Environmental Pollution 107, 263–283.
Krzyżak, J., Pogrzeba, M., Rusinowski, S., Clifton-Brown, J., McCalmont, J. P., Kiesel, A., Mangold, A., Mos, M., 2017. Heavy metal uptake by novel miscanthus seed-based hybrids cultivated in heavy metal contaminated soil. Civil and Environmental Engineering Reports 26(3), 121–132.
Lasat, M. M., 2002. Phytoextraction of toxic metals. Journal of Environmental Quality 31(1), 109-120.
Lloyd, J.R., Anderson, R.T, Macaskie, L.E., 2005. Bioremediation of metals and radionuclides. In: Bioremediation: Applied microbial solutions for real world environmental cleanup. Atlas, R.M and Philp, J.C (eds). ASM Press, Washington, D.C. pp 294.
Madeira, A.C., De Varennes, A., Abreu, M.M., Esteves, C., Magalhães, M.C.F., 2012. Tomato and parsley growth, arsenic uptake and translocation in a contaminated amended soil. Journal of Geochemical Exploration 123, 114-121
Martínez-Fernández, D., Arco-Lázaro, E., Bernal, M.P., Clemente, R. 2014. Comparison of compost and humic fertiliser effects on growth and trace elements accumulation of native plant species in a mine soil phytorestoration experiment. Ecological Engineering 73, 588-597.
McGrath, S.P., Lombi, E., Gray, C.W., Caille, N., Dunham, S.J., Zhao, F.J., 2006. Field evaluation of Cd and Zn phytoextraction potential by the hyperaccumulators *Thlaspi caerulescens* and *Arabidopsis halleri*. Environmental Pollution 141,115-125.
Mench, M., Lepp, N., Bert, V., Schwitzguébel, J.P., Gawronski, S.W., Schöder, P., Vangrosveld, J., 2010. Successes and limitations of phytotechnologies at field scale: outcomes, assessment and outlook from COST action 859. Journal of Soil and Sediment, 10, 1039–70.
Morillo, E., Villaverde, J., 2017. Advanced technologies for the remediation of pesticide-contaminated soils. Science of the Total Environment 586, 576–597.
Odukkathil, G., Vasudevan, N., 2016. Residues of endosulfan in surface and subsurface agricultural soil and its bioremediation. Journal of Environmental Management 165, 72–80.
USEPA, 2019. Technologies for Cleaning Up Contaminated Sites. United States Environmental Protection Agency. Available at: https://www.epa.gov/remedytech
Pardo, T. 2013. Recuperación de suelos contaminados por elementos traza mediante fitotecnologías de estabilización. PhD Thesis, University of Salamanca.
Pardo, T., Bernal, M.P., Clemente, R., 2017a. The use of olive mill waste to promote phytoremediation. In: Olive Mill Waste. Recent Advances for Sustainable Management. C.M. Galanakis (Ed.). Academic Press, Oxford. Chapter 9, pp. 183-204.
Pardo, T., Bernal, M.P., Clemente, R., 2017b. Phytostabilisation of severely contaminated mine tailings using halophytes and field addition of organic and inorganic amendments. Chemosphere 178, 556-564.
Pavel, P.B., Puschenreiter, M., Wenzel, W.W., Diacu, E., Barbu, C.H., 2014. Aided phytostabilization using *Miscanthus sinensis× giganteus* on heavy metal-contaminated soils. Science of the Total Environment, 479, 125–131.
Pilon-Smith, E., 2005. Phytoremediation. Annual Review of Plant Biology 56, 15-39.
Pogrzeba, M., Rusinowski, S., Sitko, K., Krzyżak, J., Skalska, A., Małkowski, E., Ciszek, D., Werle, S., McCalmont, J.P., Mos, M., Kalaji, H.M., 2017. Relationships between soil parameters and physiological status of *Miscanthus x giganteus* cultivated on soil contaminated with trace elements under NPK fertilisation *vs.* microbial inoculation. Environmental Pollution 225, 163–174.
Robichaud, K., Stewart, K., Labrecque, M., Hijri, M., Cherewyk, J., Amyot, M., 2019, An ecological microsystem to treat waste oil contaminated soil: Using phytoremediation assisted by fungi and local compost, on a mixed-contaminant site, in a cold climate. Science of the Total Environment 672, 732-742.
Soja, G., Wimmer, B., Rosner, F., Faber, F., Dersch, G., von Chamier, J., Pardeller, G., Ameur, D., Keiblinger, K., Zehetner, F., 2018. Compost and biochar interactions with copper immobilisation in copper-enriched vineyard soils. Applied Geochemistry 88, 40-48.
Su, Y.H., McGrath, S.P., Zhu, Y.G., Zhao, F.J., 2008. Highly efficient xylem transport of arsenite in the arsenic hyperaccumulator *Pteris vittata*. New Phytologist 180, 434-441.
Thijs, S., Witters, N., Janssen, J., Ruttens, A., Weyens, N., Herzig, R., Mench, M., Van Slycken, S., Meers, E., Meiresonne, L., Vangronsveld, J., 2018. Tobacco, sunflower and high biomass SRC clones show potential for trace metal phytoextraction on a moderately contaminated field site in Belgium. Frontiers in Plant Science 9, e-1879.
Vidali, M., 2001. Bioremediation. An overview. Pure and Applied Chemistry 73, 1163–1172.
Weil, R.R., Brady, N.C., 2017. The Nature and Properties of Soils, 15th Edition Global Edition. Pearson, Harlow, England.

Weyens, N., Van der Lelie, D., Taghavi, S., Newman, L., Vangronsveld, J., 2009. Exploiting plant–microbe partnerships for improving biomass production and remediation. Trends in Biotechnology 27, 591–8.

Zhang, M., Wang, J., Bai, S.H., Zhang, Y., Teng, Y., Xu, Z., 2019. Assisted phytoremediation of a co-contaminated soil with biochar amendment: Contaminant removals and bacterial community properties. Geoderma 348, 115-123.

Zhao, F.J., McGrath, S.P., 2009. Biofortification and phytoremediation. Current Opinion on Plant Biology 12, 373-380.