Potential of plants for the bioremediation of soils contaminated with persistent pollutants

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Abstract. Excessive or improper use of synthetic chemical compounds caused serious environmental problems and led to increased adverse effects on the terrestrial/aquatic ecosystem and on human health. Various anthropogenic activities have a negative influence on the environmental components by releasing solid, liquid and gaseous wastes, which contain different pollutants such as heavy metals, cyanides, petroleum hydrocarbons, hydrocarbons (polycyclic aromatic, halogenated), pesticides. The paper addresses the removal of some pollutants from the environment, namely persistent organic and inorganic pollutants by bioremediation, exploiting the ability of plants to bioaccumulate contaminants from environment. The work includes several considerations on environmental pollution with persistent pollutants, with particular emphasis on persistent organic and inorganic pollutants in European context. Further, some concern address the decontamination of soils polluted with persistent contaminants, which encompass a diversity of techniques, from simple processes to advanced engineering technologies. Since in recent years the biological methods have been taken into account being easier to implement comparative to other, conventional cleaning up methods, we discussed phytoremediation for soil cleaning up, contaminated with heavy metals or persistent organic pollutants. A special emphasis is placed on contaminants phytotoxicity and plants tolerance.
Key Words: bioaccumulation, clean-up, heavy metals, PCB153, plant, phytotoxicity, tolerance.

Environmental pollution with persistent pollutants. In recent years, rapid industrialization, urbanization, agricultural practices, improper use of chemicals and pesticides, non-qualitative irrigation water, mining, increasing quantities of waste have led to pollution of soils and water resources. Among natural resources, soil is the basic pillar that ensures the sustainability of agriculture and the support of human and animal life (Mougin 2009; Gavrilescu 2014). Unfortunately, during time and particularly in the last decades soil has been drastically affected by pollution. The pollution of the soil can occur as a result of natural phenomena, but the anthropogenic activities have led to the release of many compounds as pollutants, which due to their properties irreversibly affect the quality of the environment and induce negative impacts on the existence of terrestrial life (Clarkson 1986; Odobasic 2012).

Persistent organic pollutants (POPs) (organochlorine pesticides, polychlorinated biphenyls (PCBs), perfluorinated compounds (PFCs), polycyclic aromatic hydrocarbons (PAHs), bromide compounds (BFRs), dioxins and furans) as well as those of an inorganic nature (heavy metals, radionuclides) are often identified in soil and sediments (Wania & Mackay 1996; Gavrilescu 2005). Since the majority of them enter the food chain, these contaminants are harmful to food safety, human health and ecosystems (Caliman et al 2009; Hlihor et al 2018).

Globally it was found that over 20 million hectares of soil are contaminated by heavy metals (As, Cd, Cr, Hg, Pb, Co, Cu, Ni, Zn and Se), often the concentrations present in the soil being much higher than those imposed by towards legislation (Pandey et al 2016; Li et al 2019). Heavy metals and mineral oils contribute with nearly 60% to soil contamination, followed by polycyclic aromatic hydrocarbons (PAH), BTEX (benzene, toluene, ethylbenzene and xylene), CHC (chlorinated hydrocarbons), phenols and cyanides (Panagos et al 2013) (Figure 1).

According to recent estimates, it would be necessary to clean-up about 250,000 locations in EU member countries, and the number of sites requiring remediation will increase by 50% by 2025. However, the remediation is progressing relatively slowly over the past 30 years, since only about 80,000 sites have been cleaned (Gillespie & Philp 2013; Mougin et al 2009). In Romania, the number of contaminated sites is estimated at around 210, of which most are found in Hunedoara County (41 sites), while the counties with the least polluted sites are Călărași, Giurgiu, Olt and Tulcea (Figure 2).

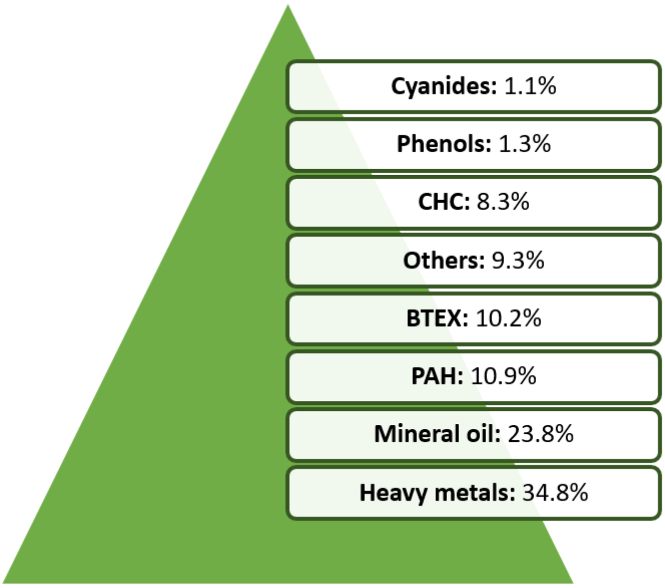

Figure 1. Classes of pollutants present in soil at European level (Panagos et al 2013).

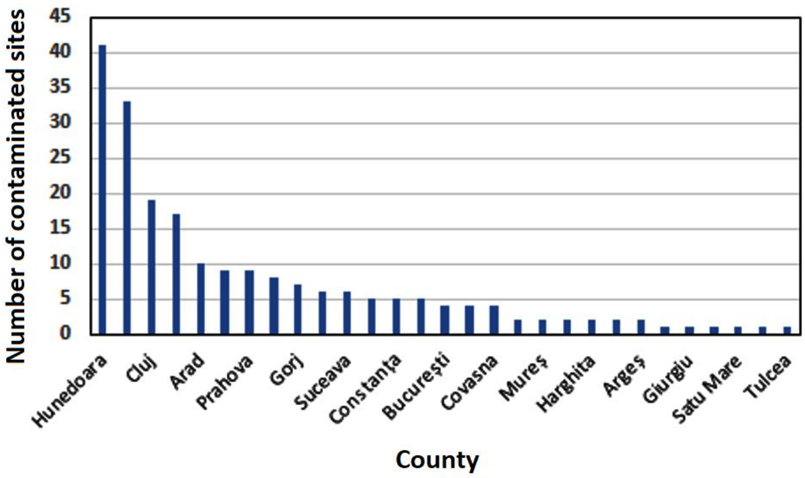

Figure 2. Contaminated sites in Romania (HG 683, 2015).

At present, a comprehensive and adequate set of soil rules has not been developed at EU level, but other legislative packages such as agriculture, water, waste, chemicals and the prevention of industrial pollution refer indirectly to soil protection. Due to their specific nature and limited reference to soil protection, these packages are not sufficient to ensure an adequate level of protection for all soils in Europe. The thematic strategy for soil protection (COM 231, 2006) points to the need for further actions to ensure high soil protection, establishing the main purpose of the strategy and the measures needed to combat the main eight soil degradation processes in the EU: erosion, reduction of organic matter content, pollution, salinization, compaction, loss of soil biodiversity, sealing, landslides and floods.

The Seventh Environment Action Program highlighted that by 2020 land should be managed sustainably, soil - adequately protected, and Member States should make considerable efforts to reduce soil erosion for increased organic matter and pollutant remediation (FAO 2015).

In Romania, the regime of persistent organic pollutants in the environment and the strategies for reducing them are regulated by the National Plan for the implementation of the Stockholm Convention on persistent organic pollutants, which presents a series of objectives for solving environmental and health problems (Caliman et al 2009). HG 1403 (2007) *"on the restoration of areas where soil, subsoil and terrestrial ecosystems were affected"* establishes the legal framework for carrying out remediation and reconstruction activities of areas where soil, subsoil and terrestrial ecosystems have been affected. HG 1408 (2007) *"on the modalities of investigation and evaluation of the pollution of the soil and the subsoil"* regulates the modalities of investigation and evaluation of the pollution in order to identify the damages and to establish the responsibilities for the restoration of the sites.

POPs and PIPs. Organic and inorganic persistent pollutants reach the ground due to industrial discharges and some inappropriate agricultural practices. The persistence of these chemicals in soil is a threat to human health and ecosystems (Huang et al 2018). Therefore, soil clean-up and pollution prevention are needed to reduce the negative impacts of persistent pollutants on the environment, being essential for increasing and maintaining a higher yield of agricultural crops (Alì et al 2013; Mougin et al 2009; Hassan et al 2017).

Persistent organic pollutants are toxic substances that reach the environment as a result of different human activities. POPs are a major problem due to their persistence, long distance transport, semi-volatility, their ability to accumulate in tissues and their extremely toxic character, even at low concentrations (Gavrilescu 2005; Xu et al 2013; Pariatamby & Kee 2016). As a result, POPs are part of the class of persistent, bioaccumulative and toxic substances (PBT substances), but they also have the characteristic of being able to be transported by air, water and migratory species across international borders, being stored away from their place of emission, where they accumulates in terrestrial and aquatic ecosystems.

POPs can be found in the gaseous, liquid or solid phase environmental components. Depending on the physical-chemical properties, they behave differently during transport: some are very volatile ("flyers") while others have a high affinity for water ("swimmers"). This is explained by the existence of a very wide range of the values of the distribution coefficients between different phases, of the POPs (Wania & Mackay 1999). Once emitted into the atmosphere, POPs are transported by air currents and then deposited on the ground or in water. Due to their persistence, some POPs may be released back into the atmosphere through volatilization. Thus, through a succession of deposition-emission-transport stages ("grasshopper effect"), POPs can be transported over long distances. Also, POPs evaporate in the atmosphere in the warm regions of the globe, they are transported by air currents to the cold regions where their condensation takes place, possible phenomenon also in the mountain regions (Yang et al 2017). Polar regions become areas where the concentration of POPs is high, due to the global condensation process (Wania & MacKay 1996; Webster et al 1998).

The ecotoxicological effects of POPs are intensively studied and are divided into: (i) effects on human health, (ii) effects on animals and (iii) effects on the environment. POPs can be carcinogenic (causes cancer), mutagens (causes genetic mutations in the DNA), teratogens (affects reproductive function), allergens (exaggerated immune response), neurotoxic (attacks central and peripheral nervous system) and endocrine disruptors (alters system function endocrine, interferes with hormones) (Directive 548 1967).

Heavy metals, such as cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), lead (Pb), nickel, zinc (Zn) and Arsenium (As) are included in the category of persistent inorganic pollutants (PIPs) used in agriculture, industry and therefore released into the environment, so that at European level there are over 10,000 sites contaminated with heavy metals (Moolenaar & Lexmond 1998; Tchounwou et al 2014).

Of the most important characteristics of metals, toxicity, biodegradability, bioaccumulation, mobility, solubility, and valence are dependent on the specific physico-chemical form in which the metal is found. Various studies have shown that the free metal hydrate ion is the most toxic form because it is much easier and faster absorbed by the cells of organisms (Odobasic 2012). Also the toxicity of these persistent pollutants on the environment depends on the concentrations detected (the maximum permissible limits varying from metal to metal), as well as on their ability to form bonds with the thiol group of proteins, thus altering the biochemical life cycle when they enter the organisms (Jaishankar et al 2014; Tchounwou et al 2012).

Metals in the soil can be found either as free ions or in combination with other components in its structure. These ions can be adsorbed by inorganic solids, they can form insoluble inorganic compounds (carbonates and phosphates), soluble metal compounds in soil solution, complex organic combinations or they can form metal silicates. Metallic silicates represent the most stable form of metals with the least negative effects on the environment compared to the other forms found in the soil (Chibuike & Obiora 2014; Gavrilescu 2014).

Thus in order to protect the health of the environment and reduce pollution, different methods (physical, chemical methods) are usually applied to decontaminate the soil, so as to be possible its further reuse for agricultural or other purposes, but most of these methods are expensive, not environmentally friendly, often adversely affecting soil properties.

Decontamination of soils polluted with persistent contaminants

Short survey on methods. Decontamination of polluted soils has been a major concern lately. Decontamination involves a variety of techniques, from simple processes to advanced engineering technologies. Techniques for remediation of contaminated soils can be classified according to the place of application (Figure 3): *in situ* (which is always done on-site) and *ex situ* (which can be off-site) and according to the nature of the processes applied (physical, chemical or (biological) (Gavrilescu 2014). Physico-chemical soil remediation methods can be applied to soils contaminated with heavy metals, but only in limited regions. The application of these methods is restricted due to the high energy consumption but also to the adverse effects on soil structure and productivity.

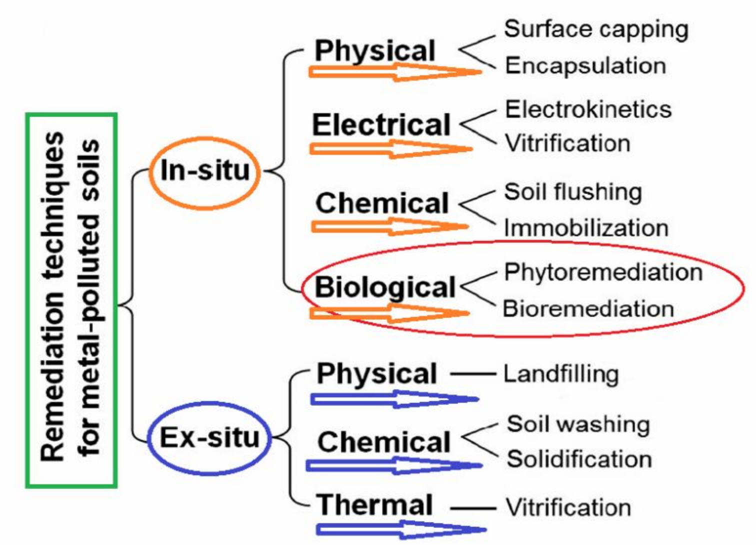

Figure 3. Remediation methods for heavy metal-contaminated soils.

In recent years, however, biological methods have been taken into account, which are easier to implement, while some estimations show that these methods have a lower cost than the conventional methods.

Phytoremediation is based on the ability of plants to absorb, accumulate, degrade the contaminants present in the soil. The remediation mechanisms are based on two basic principles, the first is the complete elimination of contaminants and the second is the transformation of contaminants into forms less harmful to the environment.

Soil phytoremediation. Phytoremediation is an ecological approach and a sustainable method for soil cleaning up compared to conventional soil remediation techniques, which exploits the ability plants to absorb and sometimes biodegrade various contaminants (Ashraf et al 2019). The concept of phytoremediation was first introduced in 1983. Phytoremediation is also called agro-remediation, botanical-remediation or green remediation, and is a good solution to treat contaminated soils. It can be applied in combination with other remediation techniques for efficiently pollutants removal.

Phytoremediation processes can be effectively applied to reduce environmental contamination as well as to prevent and control pollution. However, there are still a number of difficulties and issues that are not sufficiently elucidated and argued. The effectiveness of plants and/or microorganisms in the bioremediation process is still limited by some shortcomings caused by the toxicity of the target contaminants and by the limited ability of living organisms involved in bioremediation to deal with the toxic pollutants from the contaminated environment (Ma et al 2011; Manara 2012; Pavel et al 2013).

The efficacy of phytoremediation can be increased by the use of chelating and acidifying agents, organic chemicals and fertilizers, transgenic plants, microorganisms and the application of plant growth regulators. Recently, the use of plant growth regulators (streams, salicylic acid, cytoxins have been investigated as an appropriate method to improve the effectiveness of phytoremediation. The use of these materials increases plant biomass and reduces the negative effects of the presence of contaminants in the plant (Rostami & Azhdarpoor 2019).

Depending on the nature of pollutants and plants, several mechanisms are involved in the process of soils phytoremediation: phytoextraction, phytovolatilization, phytostabilization, phytostimulation, phyto-degradation, re-degradation (Yadav et al 2014; Yadav et al 2018).

The phytoremediation process is influenced by a variety of factors such as the characteristics of the plant used, the physico-chemical properties of the pollutants, also the soil pH, clay content, soil organic matter content, nutrients, soil moisture and temperature (Neilson & Rajakaruna 2014; Yadav et al 2018) (Table 1).

Table 1

Factors affecting phytoremediation

Factor	*Description of influence*
Plant species	- plant characteristics as hyperaccumulator; - the suitable plant species for a particular pollutant or combinations of pollutants.
Pollutant characteristics	- physical and chemical properties of organic pollutants, such as molecular mass and hydrophobicity with K_{OW} and K_{OA} parameters; - the organic pollutants with molecular mass below 1000 are readily taken up by the roots of the plants; - high K_{OW} organic pollutants are readily absorbed by plant roots, and low-K_{OA} organic pollutants are readily absorbed by plants in the air.
Soil characteristics	- soil pH, texture, moisture, cation exchange capacity, temperature and nutrient content influence the solubility and bioavailability of pollutants.
Environmental temperature	- controls the transpiration, growth and metabolism of plants and therefore directly affects both the absorption of pollutants and their elimination; - the removal rate of the metal increases linearly with increasing temperature; - soil moisture content, relative air humidity, difference in soil and air temperature, soil heat fluxes; - the different metabolic pathways of accumulator plants.

Contaminants phytotoxicity and plants tolerance. The discovery of hyperaccumulating plant species (hyperaccumulators) has revolutionized the phytoremediation technology, as these plants have the capacity to absorb metal 100 times larger than the normal plants. Hyperaccumulating capacity of plants are a natural property which makes plants capable of accumulating pollutants in tissues without developing symptoms of toxicity. There are over 450 plant species from 45 hyperaccumulating families such as Brassicaceae, Asteraceae, Caryophyllaceae, Cyperaceae, Cunoniaceae, Fabaceae, Flacourtiaceae, Lamiaceae, Poaceae, Violaceae and Euphorbiaceae. Also, the plants used in phytoremediation must meet other criteria, such as (Pantola & Alam 2014):

1. concentration of contaminants accumulated in plants should be 50 to 100 times higher than in ordinary plants;
2. bioaccumulation coefficient must have a value greater than 1;
3. concentrations of pollutants in the stems and leaves must be higher than in the roots;
4. rapid growth and high biomass;
5. easy to grow.

Plants are very different in their ability to accumulate contaminants and therefore testing and selection of plants in terms of their degree of tolerance and the required amount of biomass are essential stages (Alaboudi et al 2018). In this context, a phytoremediation study has to explore the ability of selected plants to remove target contaminants (heavy metals, persistent organic pollutants) from water and soil and plants tolerance for some toxic persistent pollutants.

Heavy metals phytotoxicity. The high concentrations of different types of heavy metals generate toxic effects, which are manifested by inhibiting cytoplasmic enzymes and deteriorating cellular structures as a consequence of oxidative stress (Assche & Clijsters 2009; Jadia & Fulekar 2009). The exchange of cations between plants and the environment is an example of the indirect toxic effect of heavy metals (Taiz & Zeiger 2002). For example, a decrease in the number of beneficial microorganisms in the soil due to a high concentration of metals can lead to a decrease in the rate of decomposition of organic matter, thus reducing the available amount of nutrients. The interference of heavy metals with the activity of microorganisms in the soil can lead to inhibition of the enzymatic activities useful for the metabolism of plants. Also, a decrease in the growth potential of the crop plants generates toxic effects (direct and indirect), sometimes causing even their death, which leads to decreased agricultural productivity. Plants can develop tolerance against heavy metal ions toxicity at different concentrations and this can be assessed by means of some indicators such as: germination index, relative growth in stems and roots, the index of root and stem inhibition. Figure 4 illustrates the inhibition index of some plants (mustard, rape, redroot) tested in the presence of Cd (II) ions (Report 2018).

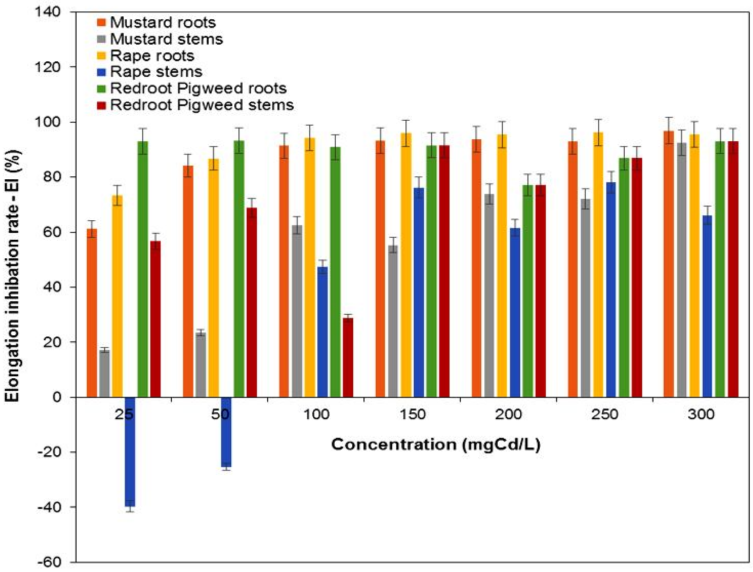

Figure 4. The inhibition index of roots and stems of some plants (mustard, rape, redroot) tested in the presence of Cd (II) ions.

A generalized dose response model for plants exposed to metals to support the explanation of the results described in Figure 4 is illustrated in Figure 5.

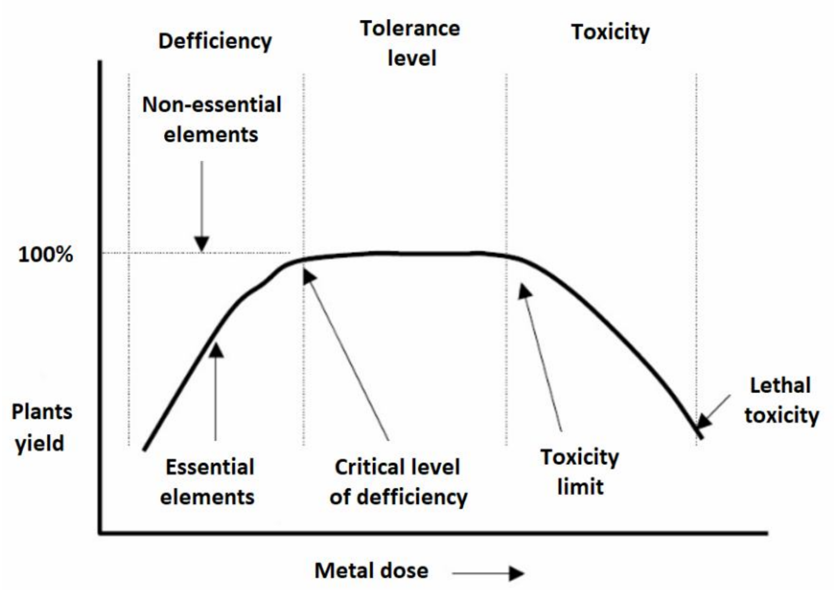

Figure 5. Dose-response curve for essential metals (Malan 1999; Madyiwa 2006).

When plants receive a high level of essential elements, such as Cu, Ni and Zn, their development efficiency increases with increasing metal dose. If the deficiency is eliminated, the accumulation and absorption of the metal will reach the critical limit, and the yield will reach a maximum level. The tolerance level will increase as the accumulation level increases beyond this level, and the further increase in the metal content does not affect the plants or their yield when the metal dose is within the tolerance range. Tolerance occurs when inactive complexes or deposits of metals are formed and therefore metals are not potentially toxic. Increasing the metal dose above the upper tolerance limit induces adverse effects on soil flora and fauna and therefore biological activity (Clarkson 1986; Madyiwa 2006). The upper limit of tolerance represents the toxic level of the metals in which the degree of absorption of the essential elements such as Cu, Ni and Zn, or of the non-essential elements such as Pb and Cd is excessive, and the result leads to adverse effects on soil biota and plants, as well as mammals, human consumers through the food chain (Moolenar & Lexmond 1999; Madyiwa 2006).

Phytotoxicity of persistent organic pollutants. Most POPs are absorbed by plants through roots and leaves and cause disruption of their growth and development when they enter the cellular tissue. Pesticides control or destroy plants through a variety of mechanisms, even by inhibiting biological processes such as photosynthesis, mitosis, cell division, enzymatic function, root growth or leaf formation, pigment, protein or DNA synthesis; destruction of cell membranes or promoting uncontrolled growth (William et al 1995; Gavrilescu 2005). Applying a pesticide can lead to toxic and rapid effects, from seed germination to plant growth, as a result of the modification of biochemical and physiological enzymatic and nonenzymatic antioxidants, which ultimately affect the production of enzymes (Hlihor et al 2018).

Polycyclic aromatic hydrocarbons (PAHs) induce growth inhibition of leaves, stems or roots in some plants, if their concentration is above the maximum tolerated concentration of the plant. They also lead to inhibition of antioxidant enzyme synthesis and detoxification process (Carlo-Rojas & Lee 2009). Polychlorinated biphenyls (PCBs), widely used in industry and hexachlorocyclohexanes (HCHs) widely used in agriculture, belong to the most representative classes of persistent organic pollutants (POPs), included in the Stockholm Convention. According to the data presented in Figure 6, a strong upshot of developing high tolerance of plants against the toxicity of PCB153 is shown by mustard and rape.

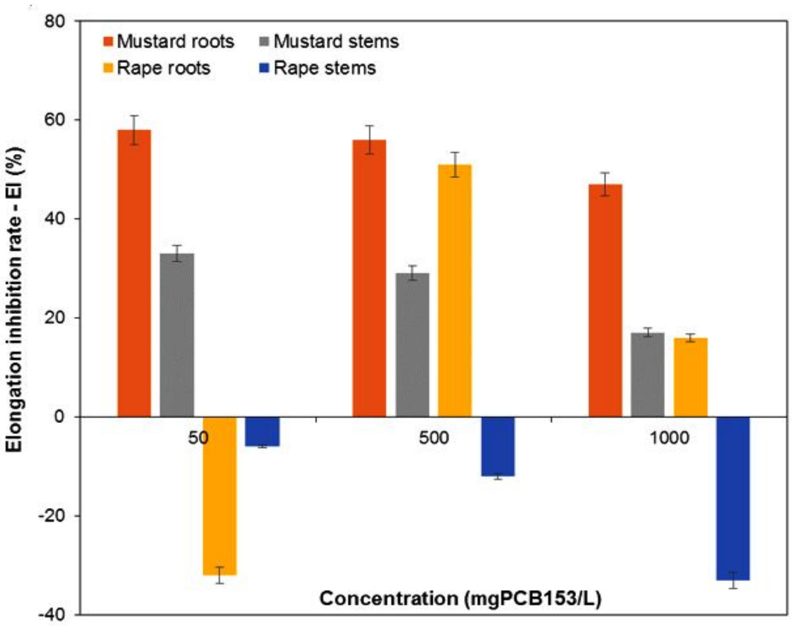

Figure 6. The elongation inhibition rate of mustards and rape roots and stems in the presence of PCB153.

Some advantages and disadvantages of phytoremediation. Phytoremediation, like any other technology used to decontaminate polluted soils has advantages, but also disadvantages. Phytoremediation is an efficient, environmentally friendly and low cost technology. It also contributes to the prevention of landscape damage, and the obtained biomass can be used to produce energy. One of the disadvantages of the phytoremediation process is the difficulty in identifying and selecting feasible plant species. Table 2 describes some advantages and disadvantages of phytoremediation.

Table 2

Advantages and disadvantages of phytoremediation (Pantola & Alam 2014)

Advantages	*Disavantages*
Environmentally friendly	It is necessary to identify and select plant species
Applied to both organic and inorganic pollutants	Properties of pollutants, bioavailability of pollutants, environmental conditions influence the phytoremediation process
Small amount of waste	Very high concentrations of contaminants can be toxic to plants
Does not require specialized equipment / personnel	Requires more seasons of vegetation
Lower costs than conventional methods	The potential for transfer of pollutants in other environments is high
Easy to implement and maintain (plants are a cheap and renewable resource)	Effective activity is limited to the roots of plants
The resulting biomass can be used to produce bioenergy	
It can be used in combination with other remedial methods	

Conclusions. Numerous pollutants (heavy metals, pesticides, dyes, polychlorinated biphenyls, petroleum hydrocarbons) reach the environment following their release from different industries. These pollutants are toxic and can have negative effects on flora, fauna, but also on human health.

Bioremediation involves the use of living organisms for removing, degrading or transforming pollutants into other forms less toxic to the environment. The use of plants for the bioremediation of soil contaminated with different pollutants is an economically, socially and environmentally reliable alternative. Both organic and inorganic pollutants present in different environments (soil, sediments, water) can be retained and/or degraded by plants. Of the mechanisms involved in phytoremediation, phytoextraction is the most efficient process for inorganic pollutants, while rhizodegradation and phytodegradation are the most efficient for organic pollutants.

The use of plants for removing pollutants (phytoremediation) is a technology that has received great attention in recent years due to lower costs compared to physical-chemical methods, but also due to their worthy ability of plants to decontaminate the polluted soil.

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