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Phytoremediation as an antidote to environmental pollution

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Introduction

The increasing contamination of the world around us with heavy metals brings the necessity to search for effective ways of its cleanup. In recent decades, particular attention has been paid to biological methods of environmental remediation. The development of these methods has been significantly improved by the understanding of how organisms react to various stressors, as well as the physiological and molecular mechanisms of toxic compounds' uptake and their neutralization by living organisms. Thus, bioremediation refers to the use of bacteria, algae, fungi and plants to remove or detoxify heavy metals, radioactive substances and diverse organic compounds that are present in polluted environments (Kumar et al. 2011, Ali et al. 2013).

Among the biological methods of environmental cleanup, phytoremediation technology can be distinguished. The term 'phytoremediation' is a combination of two words: Greek *phyton* – meaning plant, and Latin *remedium* – meaning a measure against evil. The idea of using plants to improve the quality of environment has existed since the end of the 18th century, when Joseph Priestley, Antoine Lavoissier, Karl Scheele and Jan Ingenhousz demonstrated the usefulness of plants for air purification (Rogers 2011, Passalia et al. 2017). Nevertheless, the concept of plant cover establishment on huge post-mining areas for the purposes of stabilizing them and reducing their negative visual impact only appeared in the 1980s (Barceló and Poschenrieder 2003). In this way, phytoremediation technology has emerged as a new approach to apply plants able to grow in contaminated habitats for cleaning or reducing the toxicity of standing surface waters, watercourses and groundwater, as well as soils and soil-free grounds (i.e. substrates of anthropogenic origin, such as materials generated as a result of industrial activities, that do not

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exhibit soil-specific properties) (Lee 2013, Ali et al. 2013, Hanus-Fajerska and Koźmińska 2016). Currently, this promising technology is an alternative to traditional methods of remediation which are based on changing the physical and chemical properties of soil, chemically inactivating metals, filtration or evaporation, which are all expensive and may cause secondary pollution of the environment (Mench et al. 2010).

Phytoremediation strategies

The influence of plants on toxic substances can take different forms depending on the plant species, the properties of the contaminants, or specific conditions prevailing in the polluted environment. Moreover, the mechanisms of pollutant removal or its inactivation are often very complex and require a combination of several different methods. Fundamental phytoremediation techniques can be classified based on the type of process and the features of the medium in which it takes place. Among them, phytostabilization, phytoextraction or phytovolatilization techniques can be used for the removal of heavy metals from contaminated substrates, while phytodegradation and rhizofiltration do not concern soils polluted with metallic elements (Sarwar et al. 2017). The phytoremediation methods are shown schematically in Figure 1.

Phytostabilization is a technique that allows the immobilization of contaminants in the environment, therefore limiting the transfer of toxic compounds deep into the soil profile and into the atmosphere as dust particles (Muszyńska et al. 2013b). The decrease of metal mobility occurs through the increased uptake of its ionic form and accumulation in roots, adsorption onto the root surface, or precipitation within the rhizosphere (Maestri et al. 2010). This method is also highly ranked among the known biological methods of surface stabilization in the case of waste heaps or other post-industrial areas. In this context, the aim of phytostabilization is the restoration and permanent establishment of dense vegetation cover in order to prevent erosion and minimize wind dispersion of contaminants, as well as the initiation of soil-forming processes as well as enabling and accelerating the process of natural colonization (Singh and Santal 2015, Ciarkowska et al. 2017a, Muszyńska et al. 2017).

Contrary to phytostabilization, **phytoextraction** is the removal, i.e. 'extraction', of pollutants from the solid or liquid substrates through their uptake by plant roots and further accumulation in the shoots. The success of phytoextraction depends on both the bioavailability of metal ions in the substrate and the ability of the plant to absorb them through its underground parts and the ease of translocation to its aboveground parts (Muszyńska and Hanus-Fajerska 2015, Sarwar et al. 2017). Each of these stages can significantly limit the effectiveness of phytoextraction, hence the research on this process requires cooperation of specialists in the field of soil science, microbiology, and plant physiology. Currently, this technique is the most widespread and cost-effective method of soil cleanup from heavy metals and radioactive substances. Perhaps for this reason it is often, incorrectly, considered a synonym of phytoremediation, which is a much broader concept that also includes other approaches for dealing with toxic substances.

In areas contaminated with selenium, mercury and arsenic, the technique of **phytovolatilization** (syn. phytoevaporation) can be applied. This process involves the uptake of metals from soil, their conversion to volatile forms, and subsequent release into the atmosphere in considerably less toxic forms (Sarwar et al. 2017). The best-known example of the

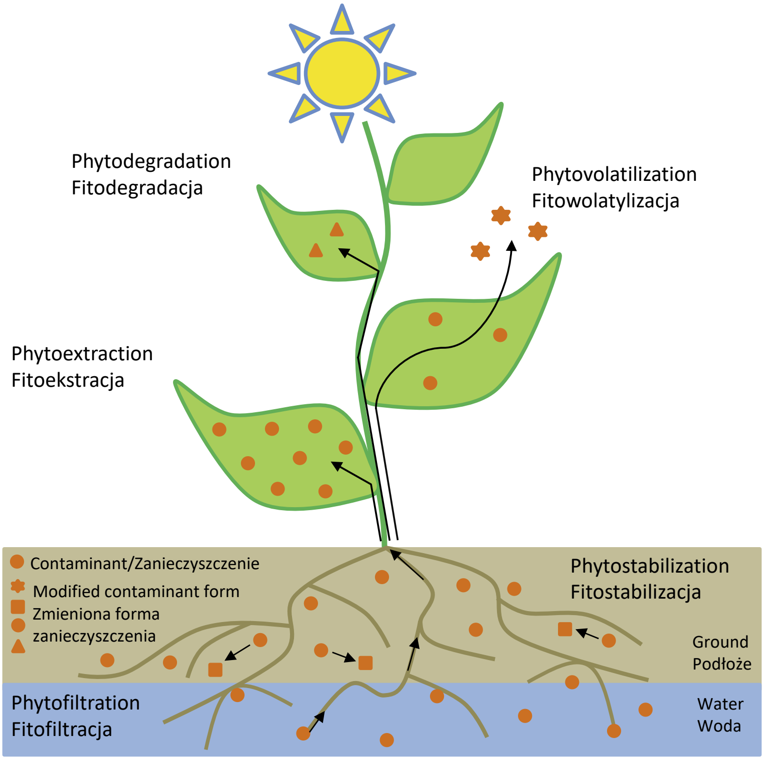


Fig. 1. Phytoremediation methods of solid and aquatic substrates contaminated with toxic substances (according to Kushwaha et al. 2016). Arrows show the direction of toxic compounds' (circles) movement leading to their accumulation or transformation within the plant organism and in the external environment: square – contaminant modified by precipitation within the rhizosphere, asterix – evaporation of contaminant into the atmosphere, triangle – decomposition of contaminat into less toxic forms within plant tissue

Ryc. 1. Metody fitoremediacji podłoży stałych i środowisk wodnych zanieczyszczonych substancjami toksycznymi (na podstawie Kushwaha i in. 2016). Strzałki przedstawiają kierunki przemieszczania się substancji toksycznych (kółka), prowadzące do ich gromadzenia lub przemian w obrębie organizmu roślinnego jak i w środowisku: kwadracik – zanieczyszczenia zmodyfikowane poprzez unieczynnienie w ryzosferze, gwiazdka – substancje toksyczne odparowane do atmosfery, trójkąt – zanieczyszczenie rozkładane do form mniej toksycznych wewnątrz tkanek roślinnych

phytoevaporation approach is the conversion of selenium to dimethylselenide or selenomethionine, which are both about 500 to 700 times less harmful than selenium in its elemental form (Jabeen et al. 2009). Such capability is characteristic of *Brassica juncea* (L.) Czern., while *Pteris vittata* L. may evaporate nontoxic forms of arsenic from its aboveground parts (Sakakibara et al. 2010). In the case of soil contaminated with mercury (Hg), the removal of those compounds is very difficult, since there are no known plant species capable of transforming this element. Nevertheless, in natural conditions, mercury conversion is carried out by numerous microorganisms. Therefore, genetic engineering methods are applied to introduce bacterial genes encoding enzymes that catalyze the reduction of Hg^{2+} to Hg^0 into plants, so that they may obtain the desirable feature. In this way, *Arabidopsis thaliana* (L.) Heynh., *Nicotiana tabacum* L., and *Liriodendron tulipifera* L. have already been modified, however these experiments have not yet advanced beyond the preliminary research stage (Koźmińska et al. 2018).

Phytodegradation (syn. phytotransformation) is the degradation of organic xenobiotics, such as aromatic hydrocarbons, pesticides, and bleaches, using the plant metabolic pathways. To achieve this, plants accumulate pollutants inside their tissues and transform them with the help of enzymes such as peroxidases responsible for the decomposition of phenolic compounds, nitrilases engaged in the hydrolysis of cyanines and aromatic compounds, or phosphatases that catalyze the breakdown of organophosphorus pesticides. The degradation of downloaded substances may be complete or partial (to carbon dioxide and water), and then the obtained products can be incorporated into plant structures, e.g. by lignification (Lee 2013). An example of this technique is the phytotransformation of highly toxic organophosphate insecticides by *B. juncea* (Rani et al. 2012).

To clean up water reservoirs from a relatively low concentration of heavy metals or radioactive compounds, **phytofiltration** can be applied, where plant roots (rhizofiltration) or seedlings (blastofiltration) are used to absorb contaminants from solution (Singh and Santal 2015, Hanus-Fajerska and Koźmińska 2016). Phytofiltration can be conducted *in situ* to remediate the contaminated surface and ground water, as well as industrial aqueous waste (for example mining water). Phytofiltration may also be used in the utilization of municipal sewage (often containing a significant amount of metals). Thus, small household treatment plants, based on selected species of *Salix* L., *Populus* L., *Phragmites* Adans., *Typha* L., *Scirpus* L. and other wetlands species, are being set up more and more often (Lee 2013). This method of phytoremediation also contributed to the effective removal of cesium and strontium from the water reservoirs after the nuclear disaster in Chernobyl. It was reported that hydroponic cultures of *Helianthus annuus* L. absorbed high amounts of cesium in the roots, while most of strontium was moved to the shoots (Gwóźdź and Kopyra 2003, Lee 2013, Hanus-Fajerska and Koźmińska 2016).

Criteria for plant selection for phytoremediation

A key factor in the usefulness of biological methods for environmental remediation is the appropriate identification of plant species that not only tolerate high concentrations of toxic substances typical for the remediated area, but are also characterized by fast growth, high biomass production, an extensive root system, and resistance to adverse habitat conditions and potential biotic stressors, such as pathogens or pest gradations. To determine if a particular plant species can be used in the phytoremediation techniques specified above, each species can be quantified by calculating the bioconcentration factor (BCF) and the translocation factor (TF). The former (BCF) expresses the ratio of an element's content in biomass to its concentration in the ground, and thus indicates the accumulation capacity of tested species. In turn, the translocation factor is defined as the ratio of an element's content in shoot tissue to their content in the roots, and therefore shows the ease of ion translocation within a plant organism. Furthermore, some phytoremediation techniques require the use of species that are easy to cultivate and harvest, and in the case of genetically modified plants they must have a new stable feature in subsequent generations (Mench et al. 2010, Ali et al. 2013, Pandey et al. 2015). In the phytoremediation of soils contaminated with heavy metals, phytostabilization together with phytoextraction are the most commonly used techniques of toxic ion removal from soils. For this reason, the selection criteria for plant species that determines their suitability for these two contrasting methods are discussed below in more detail.

Phytostabilization *versus* phytoextraction

Plant species selected for phytostabilization should be characterized mainly by a highly branched and widely distributed root system, as well as a high value of the bioconcentration factor and a low value of the translocation factor (below 1) (Ali et al. 2013). Their role in the immobilization of metals consists of, among others, the secretion of root exudates such as phenols, phytosiderophores, organic acids, which react with metal ions and precipitate them in the form of insoluble salts. Similarly, another product of the root system, such as carbon dioxide, can affect the pH of the soil, its redox potential, and thus decrease the pool of available metal ions in the soil solution (Martínez-Alcalá et al. 2016). Regardless of their ability to secrete compounds reacting with toxic ions, all plants positively influence the initiation of soil-forming processes and the restoration of biological life on degraded areas, resulting in gradual improvement of substrate quality and its protection against water and wind erosion. Therefore, phytostabilization is commonly used during biological reclamation of post-mining and metallurgy landfills (Mench et al. 2010, Muszyńska et al. 2013a, Dadea et al. 2017, Ciarkowska et al. 2017a). Apart from a significant reduction in the environmental burden of deposited waste materials, the establishment of a permanent, dense vegetation cover limits visual scars on the landscape and increases green spaces, which is especially important in post-industrial areas (Sklenicka and Molnarova 2010, Dadea et al. 2017). The role of pioneer species is the best fulfilled by legumes (Fabaceae family), which live in symbiosis with *Rhizobium* bacteria and therefore do not require nitrogen fertilization. Moreover, these legumes themselves supply the substrate (soil) with nitrogen. Species from the Fabaceae family are often combined with mixtures of grasses (*Agrostis* sp., *Festuca* sp.), that relatively quickly form the turf, and thus perform an anti erosion function (Zhang et al. 2010, Vaverková and Adamcová 2018). Apart from herbaceous plants, some trees can be successfully planted on chemically degraded areas (Muszyńska et al. 2013b). One of them is *Robinia pseudoacacia* L. with its small habitat requirements, fast growth, and ease of rapid spreading on the new area. As a legume plant, it is primarily valuable on nitrogen-free soil, including the burnt waste piles of coal, gravel and ash. Another species of great importance in the forest management of post-industrial areas is *Alnus glutinosa* (L.) Gaertn., which not only enriches the substrate with nitrogen but also provides precious and quickly decomposing litter (Claessens et al. 2010). Despite its high adaptation capacity, the best growth and development of *A. glutinosa* specimens can be achieved on wet grounds, while on dry ones it should be applied as an accompanying species for soil care (Dadea et al. 2017). Similarly, *Betula pendula* Roth is often used in afforestation of the poorest habitats on various industrial terrains due to its resistance to climate change, drought and nutrient deficiency as well as its rapid growth rate and easy spreading by self-seeding (Hanus-Fajerska et al. 2009, Vaverková and Adamcová 2018). Studies on the establishment of plants in areas contaminated with heavy metals have also examined a number of different shrub species. It was found that the most appropriate shrubs have a high shadow tolerance (since they mostly form the underbrush layer) and a great ability to adapt to soil-free grounds. Beginning with the most commonly used, the following shrubs species can be named: *Padus serotina* (Ehrh.) Borkh., *Cerasus mahaleb* (L.) Mill., *Ligustrum vulgare* L., *Cornus sanguinea* L., *Symphoricarpos albus* (L.) S. F. Blake, *Crataegus* sp., *Lonicera xylosteum* L., *Sambucus nigra* L., *Sambucus racemosa* L., *Lycium barbarum* L. (Muszyńska et al. 2013b, Vaverková and Adamcová 2018). Additionally, species with low soil requirements but high demand for insolation (sunlight) have the potential to enhance phytostabilization success. Among them are: *Hippophaë rhamnoides* L., *Physocarpus opulifolius* (L.) Maxim., *Caragana arborescens* Lam., *Rosa canina* L. and *Rosa rugosa* Thunb., *Syringa vulgaris* L. (Borghi et al 2008, Muszyńska et al. 2013b, 2014, Vaverková and Adamcová 2018). Some of the species mentioned above are expansively growing anthropophytes, which have become a permanent part of the local flora, and sometimes even dominate in large areas and threaten the local biodiversity (Szarek-Łukaszewska 2009). Therefore, during the selection of plant material, the consequences resulting from the invasion of introduced species should be taken into consideration. The novel approach in biological reclamation of destroyed habitats constitutes the methods based on native, local plants species that spontaneously appear on degraded areas (Tokarska-Guzik 2000, Tordoff et al. 2000, Szarek-Łukaszewska et al. 2009, Hanus-Fajerska et al. 2013, Pandey et al. 2015, Muszyńska et al. 2017). Although this approach is a promising solution, it is still rarely applied in Poland. Such restoration involves the permanent establishment of vegetation by planting the native metallophytes that are adapted to excess amounts of heavy metals in the soil, or by the introduction of small grasslands patches with its topsoil layer from older heaps to newly formed bare tailings (Szarek-Łukaszewska et al. 2009, Gupta et al. 2013, Muszyńska et al. 2017, Hanus-Fajerska et al. 2019). The suitability of metallophytes from the Olkusz region, which is ore-bearing, to the phytostabilization of post-flotation zinc-lead wastes has been successfully verified, among others, for *Gypsophila fastigiata* L. (Muszyńska et al. 2015), *Scabiosa ochroleuca* L. (Muszyńska and Hanus-Fajerska 2016), and *Dianthus carthusianorum* L. (Ciarkowska et al. 2017a, Muszyńska and Hanus-Fajerska 2017, Muszyńska et al. 2018a).

Contrary to phytostabilization, phytoextraction involves the mobilization of metal ions, their increased uptake by plant roots, efficient translocation via xylem, and accumulation in above-ground organs in high concentrations (Martínez-Alcalá et al. 2016). The phytoextraction potential of plant species is mainly determined by metal concentration accumulated in the shoots and the size of their biomass. Therefore, plants selected for this process should possess the following features: high value of both bioaccumulation and translocation factors (above 1), fast growth rate, production of more above-ground biomass, and an extensive root system. Moreover, they should be unattractive to herbivores in order to avoid entry of heavy metals into the food chain (Sarwar et al. 2017). Excellent candidates for phytoextraction are hyperaccumulating plants that can accumulate heavy metals in their above-ground organs at concentrations from 100- to 1,000-fold higher than those found in plants from unpolluted soils, and from 10- to 100-fold higher than the majority of plants growing on soil contaminated with heavy metals (Szarek-Łukaszewska 2014, Muszyńska and Labudda 2019). However, a small number of species considered as heavy metal hyperaccumulators have been characterized so far (approximately 720 depending on the accumulation criterion and the classification system) (Muszyńska and Labudda 2019). Although the phenomenon of hyperaccumulation is rather unusual, species with hyperaccumulating potential can be also found on waste heaps of the Olkusz region (Nowak et al. 2011). They include *Biscutella laevigata* L. (Babst-Kostecka et al. 2014, Bemowska-Kałabun et al. – Chapter 6 of this volume) as well as *Arabidopsis halleri* (L.) O'Kane & Al-Shehbaz (Verbruggen et al. 2013, Babst-Kostecka et al. 2018). Unfortunately, hyperaccumulators have limited potential for phytoremediation because most of them have slow growth rates, small biomass and shallow root systems, which significantly restricts their wide application for environmental cleanup (Gupta et al. 2013, Bulak et al. 2014, Muszyńska and Hanus-Fajerska 2015). An alternative to this sparse group of plants may be the application of species which produce more aboveground biomass, but simultaneously accumulate the heavy metals to a lesser extent. In this context, the most promising plants include various mustard species, for example *B. juncea*, which effectively removes lead, cadmium, chromium, nickel and zinc (Revati et al. 2017). Moreover, taking into account the great yield of *B. juncea*, its easy adaptation to various climatic conditions, and its root system which can grow to a depth of 50 cm, this species can be successfully used in metal extraction and prevention leaching of soluble ions to groundwater (Gwóźdź and Kopyra 2003).

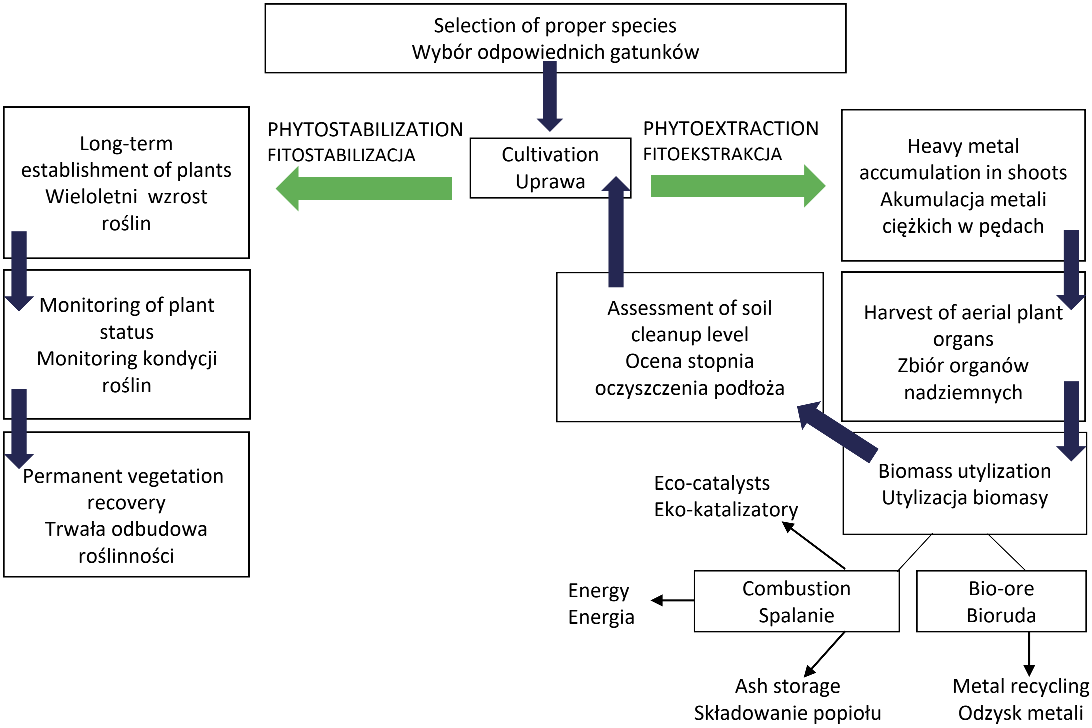


Fig. 2. The main routes of plant material treatment during phytostabilization and phytoextraction

Ryc. 2. Główne etapy postępowania z materiałem roślinnym podczas fitostabilizacji i fitoekstrakcji

The fate of plants used for phytoremediation

In addition to the undoubtable benefits of phytoextraction, how to utilize the large amount of harvested metal-rich biomass is still an open question. This problem is almost nonexistent in the case of phytostabilization, which is aimed at the permanent cultivation of plants on a degraded area. Contrary to this, plants used for phytoextraction should be removed from the purified surface at the end of growing season (Fig. 2). After harvesting, the biomass together with accumulated metals is usually combusted to obtain energy. The remaining ash might be disposed as a hazardous waste material in specialized dumps or considered as 'bio-ore' for extraction of precious and semiprecious metals (Jabeen et al. 2009, Bulak et al. 2014). This efficient and eco-friendly way of metal recovery, called phytomining, can be recommended for the mining of ores which are unprofitable when using conventional methods or are from places with too low concentration of elements to open traditional mines (Mench et al. 2010, Muszyńska and Hanus-Fajerska 2015). As an example, the cultivation of the nickel hyperaccumulators *Alyssum murale* L. and *Alyssum corsicum* Duby for the extraction of this metal provides biomass containing about 400 kg of nickel per hectare. Considering the low production costs and relatively high prices of nickel, phytomining of this element becomes financially rewarding (Chaney et al. 2007). An interesting and innovative approach to the use of hyperaccumulators biomass is the preparation of 'eco-catalysts' (Fig. 2). In this novel technology, the shoots of *Anthyllis vulneraria* L. and *Noccaea caerulescens* (J. Presl & C. Presl) F. K. Mey. containing 120,000 mg/kg of Zn can be treated as good resources of new generation catalysts for various chemical transformations (Escande et al. 2014, 2015).

Strategies for increasing plant phytoremediation potential

Genetic modification

Genetic engineering methods are more and more often used to enhance the phytoremediation potential of plants and the possibility of their application in particular environmental purification techniques. Although research on transgenic plants (genetically modified) is only in the phase of laboratory experiments, they can still significantly contribute to the development of new phytoremediation methods (Singh and Santal 2015).

Genetic engineering is primarily progressing in two main directions. On the one hand, experiments are carried out to enhance the general stress resistance of plants to heavy metals by modifying genes involved in defense reactions, in particular DNA repair mechanisms and the removal of free radicals and reactive oxygen species (Faè et al. 2014, Charfeddine et al. 2017). On the other hand, there is lots of research on the modifications of hyperaccumulating plants in order to accelerate their growth rate and intensify plant biomass accretion, as well as research on the modifications of crop plants with rapid growth to increase their ability to uptake, translocate and sequestrate metal ions (Shen et al. 2011, Shim et al. 2013, Pavlikova et al. 2014, Vrbova et al. 2015, Koźmińska et al. 2018).

Substratum amendments affecting plant growth conditions or/and metal bioavailability

Soils requiring remediation, beyond the high pollutant contents, are usually poor in nutrients and have improper physical properties, such as a lack of aggregation, which results in impermeable layers, low available water capacity, and improper soil air composition. Therefore, plants should produce large biomass in a short time in order to perform a successful remediation (i.e. significantly decrease pollutants in a reasonable period of time). For this reason, the addition of organic matter and/or mineral nutrients to soils in the form of soil amendments is recommended to improve physical, chemical and biological soil properties, and thus stimulate plant growth. In other words, the exploitation of soil amendments in the course of the phytoremediation scheme leads to enhanced efficiency of the cleanup process (Weber et al. 2007, Mench et al. 2010, Ciarkowska et al. 2017b).

When growing conditions are poor, high amounts of nutrients provided in easily accessible forms helps plants to survive (Muszyńska et al. 2013). Following this rule, the oldest and most often used soil amendment is nitrogen, phosphorus and potassium fertilizer applied in two- or even three fold higher doses than in normal field growing conditions. In such cases, another important soil component is necessary, namely organic matter, which in addition to releasing nutrients constitutes a beneficial habitat for microorganisms and soil fauna determining soil biological activity (Ciarkowska et al. 2017a). The decomposed soil organic matter, called humus, is a source of nutrients but is very expensive to produce. Numerous studies have been also carried out on other organic products, mainly by-products of different industries, applied as soil amendments. An important product of this type is sewage sludge. For reclamation purposes, the municipal sewage sludge is applied because its neutral pH, high content of macroelements (i.e., organic carbon, nitrogen, phosphorous and magnesium), as well as relatively low heavy metal content makes it useful as fertilizer. Furthermore, the high organic matter content leads to the formation of organometallic bonds, resulting in a decreased bioavailability of heavy metals such as zinc, lead and cadmium, and thus supports the process of plant renewal and establishment on degraded areas (Mench et al. 2010, Bolan et al. 2014). Additionally, the sewage sludge application significantly improves the total water retention in the soil available to plants. This is of particular importance for sandy and permeable soils since it can considerably elevate the percentage of seed emergence or facilitate plant maintenance on remediated areas (Weber et al 2007, Singh and Agrawal 2008). The effectiveness of substrate supplementation with humic substances, components of humus such as humic acids, is also quite well recognized because they are key components of soil fertility (Arjumend et al. 2015). One of the sources of humic acids may be lignite which has become the basis for the composition of an organic fertilizer called Rekulter®. The application of Rekulter® significantly enhances organic carbon content in substrate and improves its sorption capacity, which results in the immobilization of pollutants and increases the quality of remediated soil (Maciejewska and Kwiatkowska 1998, Ciarkowska et al. 2017b). Sugar beet residues (SBR), from *Beta vulgaris* L. cultivation, have also been proposed as soil amendments with very good properties. SBR is a mainly lignocellulosic material, rich in with polysaccharides and available phosphorus, that is obtained as a by-product during the preparation of the sugar extract, followed by fermentation of obtained biomass by properly selected *Aspergillus niger* Tiegh fungi isolates. Its positive influence on phytoextraction effectiveness can be reflected in the improvement of rhizospheric microbiota biomass, the increased activity of soil enzymes – hydrolases and oxidoreductases, the stimulation of plant growth and development, as well as the stability of soil aggregates. Moreover, the by-product of sugar production, called molasses, can be served as a source of organic carbon, minerals and vitamins for soil microorganisms. Therefore, molasses application leads to increased densities of different bacterial strains, and in this way influences the soil quality (Wiszniewska et al. 2016). To promote plant growth and development on degraded areas, paper industry wastes are also considered. These amendments improve growth conditions in terms of the carbon to nitrogen ratio, as well as the water holding capacity. Furthermore, these waste materials are exploited for reducing the bioavailability of toxic elements. In recent decades, biochar utilization has been also recognized to improve soil properties. It is the solid product obtained through pyrolysis of waste biomass residues from agricultural and forestry production. Biochar may play an important role as a surface adsorbent because of its relatively structured carbon matrix, high degree of microporosity, extensive surface area, and high pH and cation exchange capacity. However, it should not be forgotten that biochar application may change the pH of contaminated soil to alkaline, and thus the chemical sorption of metals occurs. Such metal precipitation limits its utilization for phytoextraction purposes. On the contrary, biochar can significantly increase the effectiveness of the phytostabilisation of polluted matrix.

Possibilities for the application of native calamine species for phytoremediation – a presentation of our own research on *B. laevigata*

Considering the benefits of phytoremediation, the authors of this chapter attempt to select the appropriate plant species that are able to grow on post-flotation waste generated during zinc-lead ore exploitation in order to its stabilization and initiation the soil-forming processes. Particular attention is paid to native plant species that spontaneously appear on areas contaminated with heavy metals. Therefore, seed samples collected from specimens growing on old waste heaps are used to start experiments conducted in *in vitro* cultures as well as in greenhouse and field conditions (Ciarkowska and Hanus-Fajerska 2008, Muszyńska et al. 2013a, Muszyńska et al. 2018a, b, Hanus-Fajerska et al. 2019). In the first step, laboratory studies are carried out to develop a better understanding of plant adaptation mechanisms to heavy metal stress. Such approach also enables the preliminary selection of species suitable for direct implementation on zinc-lead post-flotation settler and other chemically degraded terrains (Ciarkowska and Hanus-Fajerska 2008, Muszyńska et al. 2017, Muszyńska et al. 2018b, Muszyńska et al. 2019). Given that *B. laevigata* grows naturally on old calamine waste heaps and exhibits a tolerance not only to heavy metals but also to water and nutrient deficiency (Bemowska-Kałabun et al. – Chapter 6 of this volume), this species has gained our special interest. In the first stage of our research, aseptic cultures of *B. laevigata* were established from seeds harvested from specimens growing in the old waste heaps near Bolesław (Fig. 3A), and then *in vitro* selection on media enriched with combinations of heavy metals occurring in this industrial area was accomplished (Fig. 3B–C) (Hanus-Fajerska et al. 2012, Muszyńska and Hanus-Fajerska 2012). The obtained micro-plants were successfully acclimatized to greenhouse conditions (Fig. 3D) and were subsequently used to set up both a three-year pot (Fig. 3E) and a field experiment (Fig. 3F), in which the potential of the species for stabilization of zinc-lead wastes was examined (Ciarkowska et al. 2017a, Muszyńska et al. 2017). In this part of the long-term study, *B. laevigata* was cultivated in waste material with mineral fertilization or with addition of sewage sludge as soil amendments. After three years of pot cultivation, an

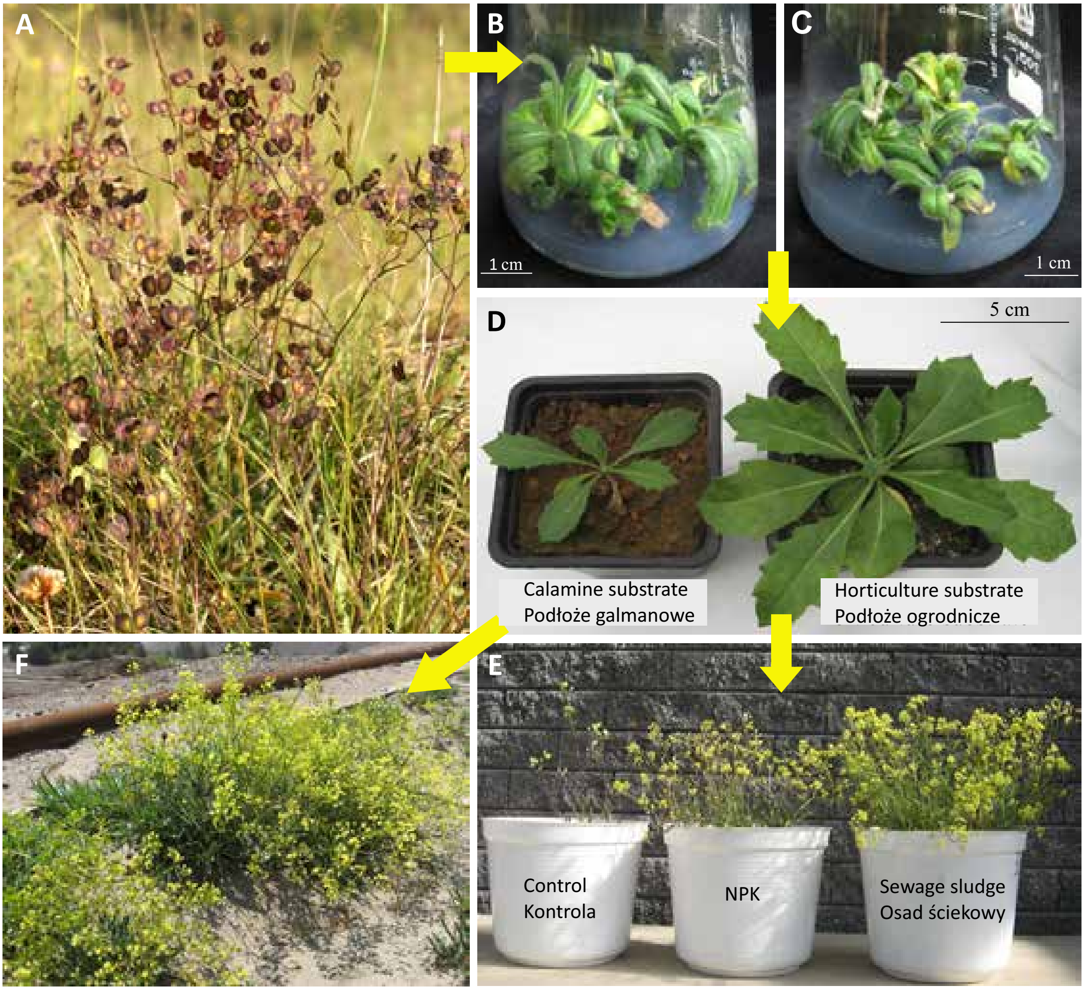
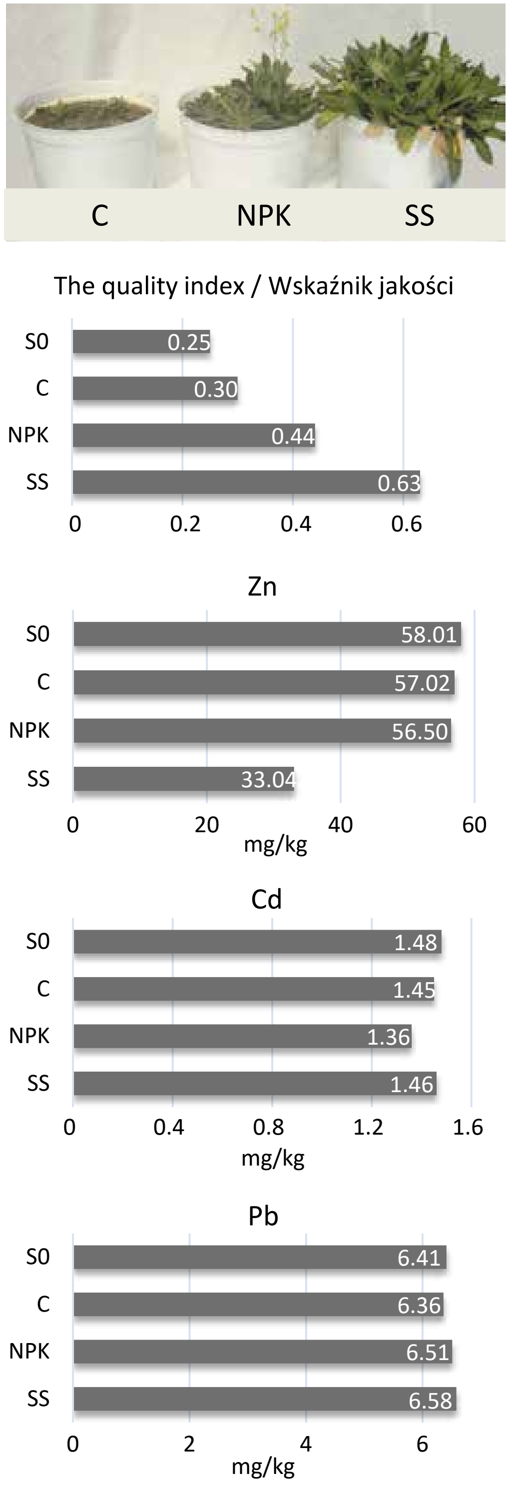


Fig. 3. The research stages on *Biscutella laevigata* usefulness for phytostabilization of post-flotation tailings: silicula fruits collection from individuals growing in natural conditions (A), aseptic culture establishment and *in vitro* selection (B–C), acclimatization to greenhouse conditions on calamine and horticulture substrates (D), pot experiment treatments (E), field cultivation (F)

Ryc. 3. Etapy badań nad wykorzystaniem *Biscutella laevigata* do fitostabilizacji odpadów poflotacyjnych: zbiór łuszczynek (owoców) z osobników rosnących w warunkach naturalnych (A), założenie aseptycznych kultur i selekcja *in vitro* (B–C), aklimatyzacja do warunków szklarniowych na podłożu galmanowym i ogrodniczym (D), obiekty doświadczenia wazonowego (E), uprawa polowa (F)

increase in soil enzyme activity and nutrient level, accompanied by the significant decrease of soluble zinc and cadmium forms in the substratum was observed particularly on post-flotation waste enriched with sewage sludge (Fig. 4). This relationship was not ascertained for lead contamination (Fig. 4), since only a small percentage of lead content occurs in the forms available for plants (Muszyńska and Labudda 2019). To conclude this experimental step, it was shown that the cultivation of *B. laevigata* in the presence of sewage sludge as an organic soil amendment can be an effective method of stabilizing wastes highly polluted with heavy metals, and contribute to the improvement of substrate quality, even if toxic ions are present (Ciarkowska et al. 2017a).

Similarly to the previous results, during field cultivation on the post-flotation settler it was found that the plant material developed and grew successfully in field conditions when post-flotation wastes were enriched with mineral fertilizers or sewage sludge (Fig. 5). The application of above-mentioned amendments also had positive effects on the physiological status of plants. This was confirmed by the analysis of commonly used markers of metabolic changes occurring in plant cells under the influence of stress factors, such as energy flow through photosystem II (Fv/Fm, PI) and the content of photosynthetic pigments (Table 1). Although *B. laevigata* specimens cultivated in the presence of sewage sludge achieved the highest photosynthetic efficiency in comparison to other treatments, in the later stages of growing season they were much more susceptible to abiotic stresses, for example light intensity or



←

Fig. 4. The pot experiment on *Biscutella laevigata* – substrate characterization before planting (the initial substrate) as well as after three years of plant cultivation. The quality index, Zn – zinc content, Cd – cadmium content, Pb – lead content, S0 – the initial substrate, i.e. post-flotation tailings before experiment establishing, post-flotation tailings at the end of plant growth: C – control (without any additives), NPK – wastes enriched with nitrogen, phosphorous, potassium, SS – wastes enriched with sewage sludge, (according to Ciarkowska et al. 2017a)

Ryc. 4. Eksperyment wazonowy na *Biscutella laevigata* – charakterystyka podłoża przed wysadzeniem roślin (podłoże inicjalne) oraz po trzech latach uprawy roślin. Wskaźnik jakości podłoża, Zn – stężenie cynku, Cd – stężenie kadmu, Pb – stężenie ołowiu, S0 – podłoże inicjalne, czyli odpad poflotacyjny przed założeniem doświadczenia, odpady poflotacyjne po zakończeniu wzrostu roślin: C – kontrola (bez dodatków), NPK – wzbogacone w azot, fosfor, potas, SS – wzbogacone w osady ściekowe, (za Ciarkowska i in. 2017a)

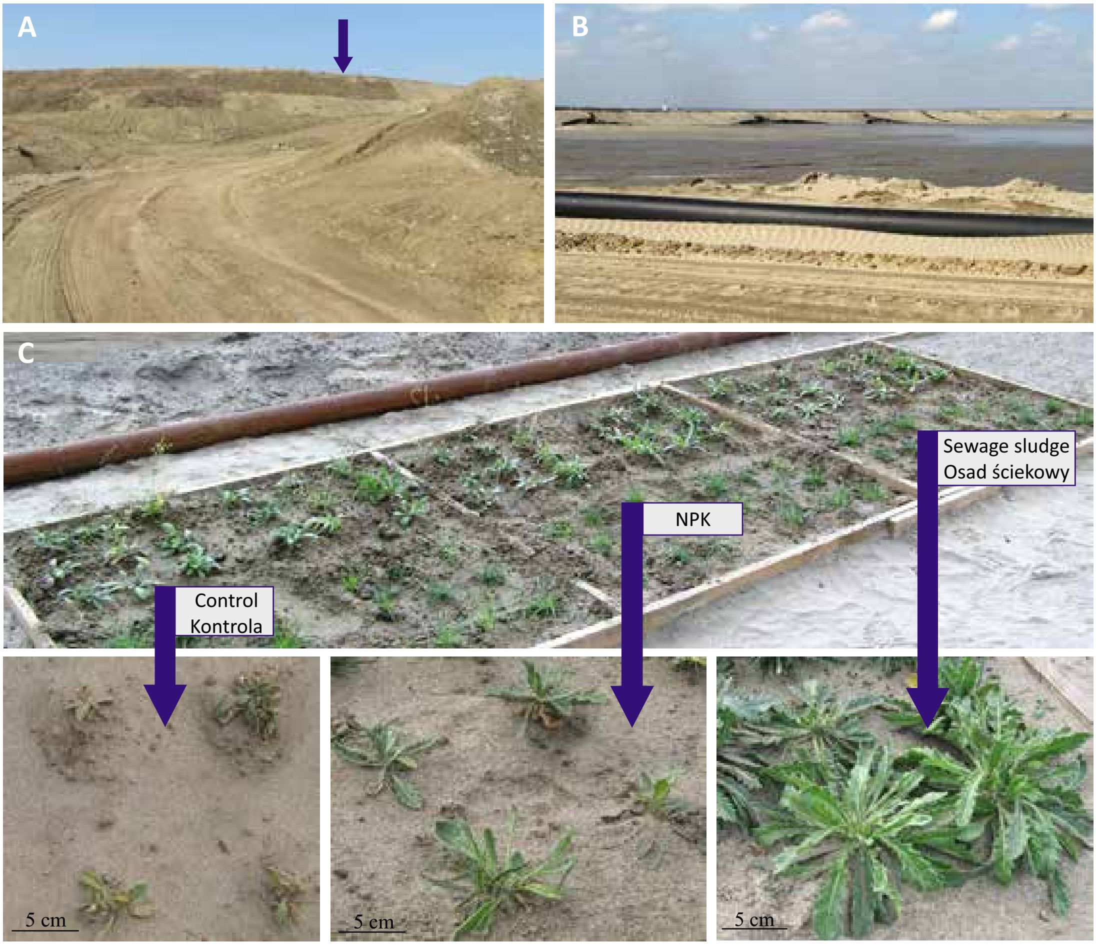


Fig. 5. The field experiment on *Biscutella laevigata* – localization of the plot on the post-flotation tailings settler (A) near the settling pond (B) as well as specimens growth on post-flotation tailings (C): without any additives (control), enriched with nitrogen, phosphorus and potassium (NPK) or with sewage sludge

Ryc. 5. Eksperyment polowy na *Biscutella laevigata* – położenie poletek doświadczalnych na osadniku odpadów poflotacyjnych (A) w pobliżu stawu osadowego (B) oraz wzrost roślin na odpadach poflotacyjnych (C): bez dodatków (kontrola), wzbogaconych azotem, fosforem i potasem (NPK) oraz osadem ściekowym

sudden temperature drop (Muszyńska et al. 2017). Nevertheless, that did not affect their ability to regenerate in subsequent years of cultivation. Therefore, it has been experimentally proven that the buckler mustard can be applied to initialize soil formation and accelerate plant succession on waste material, which is otherwise an extremely unfavourable environment for vegetation.

Perspectives of environmental purification by biological methods

Purification of the environment with plants or other biological activities limiting the spread of pollutants can be applied both as separate techniques or as supporting tools for conventional remediation methods. Contrary to conventional techniques of soil cleanup,

Table 1. Content of photosynthetic pigments [mg/g f.w.], the ratio of chlorophyll a to chlorophyll b, and parameters of chlorophyll fluorescence a (Fv/Fm and PI) of *Biscutella laevigata* during field cultivations on post-flotation tailings without any additives (control) and with mineral (NPK) or organic (sewage sludge) fertilizers. Fv/Fm – the maximum photochemical efficiency of photosystem II, PI – vitality index of photosystem II. The same letters in rows indicate that means do not differ statistically (according Muszyńska et al. 2017)

Tabela 1. Zawartość barwników fotosyntetycznych [mg/g św.m.], stosunek chlorofilu a do b oraz parametry fluorescencji chlorofilu a (Fv/Fm i PI) u *Biscutella laevigata* podczas polowej uprawy na podłożu poflotacyjnym bez dodatków (kontrola) oraz z domieszką nawozu mineralnego (NPK) lub organicznego (osad ściekowy). Fv/Fm – maksymalna wydajność kwantowa PSII, PI – indeks witalności fotosystemu II. Te same litery w wierszach oznaczają brak różnic statystycznie istotnych pomiędzy wartościami (według Muszyńska i in. 2017)

Parameter Parametr	Wastes Odpady		
	Control Kontrola	Mineral fertilizer (NPK) Nawóz mineralny (NPK)	Organic fertilizer (sewage sludge) Nawóz organiczny (osad ściekowy)
Chlorophyll a [mg/g f.w.] Chlorofil a [mg/g św.m.]	0.24 c	0.39 b	0.54 a
Chlorophyll b [mg/g f.w.] Chlorofil b [mg/g św.m.]	0.06 c	0.10 b	0.15 a
Total chlorophylls [mg/g f.w.] Suma chlorofili [mg/g św.m.]	0.30 c	0.49 b	0.69 a
Carotenoids [mg/f.w.] Karotenoidy [mg/g św.m.]	0.11 c	0.15 b	0.18 a
Chlorophyll a/b Chlorofil a/b	4.07 a	4.03 a	3.72 b
Fv/Fm	0.79 b	0.82 a	0.73 c
PI	0.98 b	1.68 a	0.90 c

this eco-friendly technology does not usually use any additional metal extracting agents, which significantly reduces the generation of secondary contamination (Mench et al. 2010). Importantly, the removal of toxins is accompanied with the improvement of soil quality and increased diversity of plant species composition, as well as the abundance and activity of soil microorganisms, which in turn have a positive effect on soil fertility and limits surface erosion processes (Ciarkowska et al. 2017a). Moreover, phytoremediation techniques are easier to perform compared to conventional methods and do not require highly specialized equipment (Ali et al. 2013). From an economic point of view, phytoremediation is also very cost-effective. For example, cleaning 1 m^3 of soil with physical methods costs from 100 to 500 USD, whereas phytoextraction of the same volume is an expense of about 50 USD (Gwóźdź and Kopyra 2003). In addition, this 'green technology' overcomes visual problems and allows a quick improvement of the aesthetic values of local landscapes. Therefore, this idea of environmental purification is widely accepted by the public (Vaverková and Adamcová 2018).

Although phytoremediation is a promising approach for the remediation of heavy metal-contaminated areas, it also suffers from some limitations. One of the basic disadvantages of phytoremediation is the relatively long time required for purification, which can take up to several years (Jabeen et al. 2009). Moreover, the substrate can only be cleaned to the depth of the root system penetration, which in herbaceous plants is a maximum length of 120 cm, while in trees it is up to 7 m (Tordoff et al. 2000, Singh and Santal 2015). Additionally, the presence of multiple types of toxic compounds may pose a challenge, since their complex interaction can make cleaning more difficult. The biological reconstruction of chemically degraded terrain is also hindered by unfavorable environmental conditions, such as high insolation, drought, low nutrient content and phytotoxic concentration of heavy metals in the ground. These factors significantly impact the effectiveness of long-term establishment of dense vegetation cover during phytostabilization and the rate of pollutant removal by phytoextraction. Phytoremediation efficiency is also strongly influenced by the limited number of plant species that are able to grow in the presence of excess concentration of toxic compounds, as well as a low resistance of plants used, if inappropriate species were selected for this process. Another danger is the spreading of compounds accumulated by plants into the food chain, which may consequently lead to the contamination of entire trophic networks. In many cases, the direction of biochemical transformations of toxic substances accumulated in plant tissues is unknown, and thus the biological properties and potential harmfulness of the metabolites formed remain ambiguous (Mench et al. 2010).

The difficulties in the commercial application of phytoremediation mentioned above show how many aspects still need to be clarified in this progressive 'green technology'. Currently, research on phytoremediation is aimed at the systematic screening of plant species and genotypes with predispositions for utilization on chemically degraded areas. The proper approach involves locally occurring native plants that have adapted through microevolutionary changes under stress conditions (Tordoff et al. 2000, Muszyńska et al. 2013a, Panday et al. 2015, Ciarkowska et al. 2017a, Muszyńska and Hanus-Fajerska 2017). The optimization of phytoremediation technology is possible with the use of various biotechnological methods. In this context, *in vitro* techniques are an innovative tool that can be exploited in both basic and applied research (Wiszniewska et al. – Chapter 8 of this volume). On the one hand, they can be used as a model system to study the metabolic pathways of heavy metal accumulation and detoxification, and on the other hand they allow the intensive vegetative propagation of valuable genotypes with the potential to be applied in phytoremediation (Doran 2009, Rai et al. 2011, Wiszniewska et al. 2017, Muszyńska et al. 2018a, b, Hanus-Fajerska et al. 2019). Simultaneously, *in vitro* selection or genetic modification may broaden the spectrum of plants that are suitable for phytoremediation, and thus help to overcome the drawbacks of this technology. Considering the ceaseless improvement of research techniques and the worldwide trend of scientific knowledge integration, it is highly probable that many questions about the commercial use of plants for environmental clean-up will be answered in the near future.

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