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Phytoremediation as an approach to clean up contaminated soil, including petroleum product contamination

Fitoremediacja jako strategia oczyszczania gleby z zanieczyszczeń, w tym zanieczyszczeń substancjami ropopochodnymi

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ABSTRACT: Contamination of the natural environment with crude oil and its byproducts is an increasing problem which requires immediate and effective action. With the higher demand for hydrocarbons, the amount of resources being extracted, transported, and stored has grown significantly. The main types of removal involve mechanical, chemical, and biological methods. Currently, the most commonly used biological approach relies on microbial – mainly bacterial – abilities to degrade toxic substances. However, studies indicate a significant impact of phytoremediation processes on contamination disposal. Several phytoremediation strategies are applied to remove various xenobiotics from the environment, namely, phytostabilization, phytodegradation, phytoevaporation, phytoextraction, and phytostimulation. More and more attention is being paid to the cooperation between plants and other organisms, primarily bacteria and fungi. The identification of microorganisms that play a key role in supporting the proper development, growth, and functioning of plants in a hostile environment is very important. The use of natural interdependencies occurring in the plant–microorganism system can be an excellent alternative to the more invasive remedial options (mechanical or chemical) available. The effectiveness of phytoremediation treatment depends mainly on factors such as environmental conditions, the species of plant and microorganisms, and the type of contamination. Biological treatment is recognized by many scientists as one of the most valuable trends in contemporary environmental protection and ecosystem renewal. Due to the proven harmfulness of some hydrocarbons, it is very important to find and develop the most efficient and cost-effective methods of cleaning up different habitats. Phytoremediation can be used as an independent process or as a complementary element to other remediation methods.

Key words: phytoremediation, crude oil, oil derivatives, plant, microorganisms.

STRESZCZENIE: Zanieczyszczenie środowiska przyrodniczego ropą naftową oraz produktami jej obróbki stanowi coraz większy problem, zmuszający do podejmowania natychmiastowych skutecznych działań. Wraz ze zwiększonym popytem na ropę oraz jej pochodne ilość związków wydobywanych, przetwarzanych, transportowanych oraz magazynowanych również wyraźnie wzrosła w ostatnich dekadach. Do głównych metod rekultywacji skażonego środowiska należą metody mechaniczne, chemiczne oraz biologiczne. Obecnie jednym z najczęściej stosowanych podejść biologicznych jest wykorzystanie naturalnych zdolności mikroorganizmów, głównie bakterii, do rozkładu substancji toksycznych, jednakże liczne badania wskazują na znaczną efektywność również procesów fitoremediacji w usuwaniu różnego rodzaju ksenobiotyków. Fitoremediacja obejmuje rozmaite techniki, mianowicie: fitostabilizację, fitodegradację, fitoewaporację, fitoekstrakcję oraz fitostymulację. Coraz większą uwagę poświęca się zagadnieniu współpracy pomiędzy roślinami a innymi organizmami, przede wszystkim bakteriami i grzybami. Identyfikacja mikroorganizmów pełniących kluczową rolę we wspieraniu prawidłowego rozwoju, wzrostu oraz funkcjonowania roślin w nieprzyjaznym otoczeniu jest bardzo istotnym aspektem badań. Wykorzystanie naturalnych współzależności występujących pomiędzy rośliną a mikroorganizmami może stanowić doskonałą alternatywę dla znacznie bardziej inwazyjnych metod stosowanych obecnie (np. mechanicznych lub chemicznych). Efektywność zabiegów fitoremediacji w dużej mierze zależy od takich czynników jak: rodzaj skażenia, czynniki środowiskowe, typ roślin oraz mikroorganizmów. Metody biologicznego oczyszczania skażonego środowiska uznawane są przez wielu naukowców za jeden z najważniejszych kierunków we współczesnej ochronie środowiska oraz odnowie ekosystemów. Ze względu na udowodnioną szkodliwość niektórych węglowodorów znalezienie i opracowanie coraz bardziej skutecznych oraz opłacalnych ekonomicznie rozwiązań remediacji zróżnicowanych siedlisk jest niezwykle istotnym trendem biotechnologii i ochrony środowiska. Fitoremediacja może być stosowana jako niezależny zabieg, a także jako element uzupełniający innych strategii rekultywacyjnych.

Słowa kluczowe: fitoremediacja, ropa naftowa, substancje ropopochodne, rośliny, mikroorganizmy.

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Introduction

Along with the development of civilization, the interference of humans in the natural environment has also increased. The benefits of petroleum hydrocarbons to human activity (such as industrial applications and technological development) have led to a higher demand for oil products. In turn, their extensive use carries many environmental threats. They can lead to contamination during oil exploitation, production, processing, and transport – and, consequently, to the generation of waste. Therefore, a substantial number of reported failures, accidents, and releases of oil-derived products to the environment has been observed. There has been a tightening of international regulations in this field in recent years. International agreements have been developed according to the guidelines of the United Nations (UN).

The structure of changes and the degree of degradation of natural conditions caused by petroleum substances expand with increased levels of urbanization. The huge impact on the natural environment is mainly associated with industrial development, motor vehicles, the development of local and international communication routes, and the growing number of places distributing fuel. Moreover, there is a substantial amount of potentially hazardous compounds in facilities and warehouses. The most common causes of pollution are unusually onerous and often uncontrolled spills of oil and petroleum compounds. In developing countries and countries with low ecological awareness, an excessive proportion of petroleum-derived or oil-contaminated waste is observed in most landfills. This practice is often out of the control of local authorities or international organizations.

The uncontrolled outflow of such substances into the environment may occur and lead to the contamination of surface and underground waters. Any oil or petrol spills easily penetrate soils and surface waters and are even found in deep groundwater resources. Due to physicochemical properties, light hydrocarbons may evaporate into the atmosphere or undergo natural biodegradation, whereas heavier hydrocarbons are retained in the soil complex and saturate the top layer. The biological regeneration of such saturated layers is very difficult. Additionally, pollutants are often transformed into other compounds, which in turn may also be harmful. These metabolites may impact the environment in ways that are unpredictable and difficult to assess, so counteraction and effective removal are a challenge. Hydrocarbon contamination is a real threat to the life and health of the population which inhabits the surrounding area, since the quality of soil, air, and water (including drinking water reservoirs) is decreased. It affects plants, animals, and humans, causing a specific impact on all levels of the trophic chain (Errington et al., 2018).

Sources of oil pollution

Crude oil and its derivatives are currently among the main pollutants of water and soil in Poland (Podsiadło and Krzyśko-Łupicka, 2013). The intensification of industrial development, agricultural economy, and population growth, along with a simultaneous increase in the demand for chemical products in recent years, have brought a significantly higher risk of the outflow of petroleum and its byproducts. These processes have led to a progressive degradation of the natural environment. (Ali et al., 2013; Feng et al., 2017). The high level of both industrialization and automotive development are the main reasons behind the growing demand for petroleum products. At the same time, the energy consumption from these sources leads to an increase in pollutant emissions.

There are several anthropogenic sources causing hydrocarbon contamination of the environment, such as:

- the exploration, exploitation, and transport of crude oil;
- the production and distribution of petroleum substances;
- the storage of oil and its compounds;
- refineries and petrochemical plants;
- machine, rail, air, and sea transport;
- the industrial use of petroleum and its byproducts;
- the municipal use of petroleum and its byproducts;
- military bases, and
- waste generated by the oil and gas industry.

Uncontrolled short-term and long-lasting spills of crude oil and petroleum products pose a danger to ecosystems. These mixtures may have very complex compositions, and their main components (hydrocarbons) reveal heterogeneous chemical structures and properties. The multitude of processes and reactions is necessary to obtain required petroleum fractions and substances (Gałązka and Gałązka, 2016; Shahsavari et al., 2017). During processing, a great number of various compounds are produced in diversified and extensive installations in refineries. Crude oil is subjected to distillation, thermal or catalytic cracking, hydrocracking, chemical, adsorption, and solvent refining, and hydrotreating as well as the mixing of individual products to enrich them or to obtain a desired compound (Gierak, 1995; Jaworska, 2012). All of the above-mentioned processes are burdened with the risk of breakdown, during which leakage may occur with the contamination of the soil and surface and underground water (Jaworska, 2012).

A consequence of the increasing amount of chemical and biological substances released by the industry and households is their impact on all elements of the ecosystem: soil, water, and air (Karczewska, 2012). Petroleum products may disturb the proper functioning of individual species as well as the entire ecosystem in contaminated areas (Radwan et al., 2012). Any disturbances of the biochemical economy and an excessive inflow of harmful compounds will cause changes in the balance of the system between populations of individual organisms living in a given habitat (Puchalska, 1999; Traczewska, 2011; Karczewska, 2012). Pollution may also lead to the extinction of a given habitat.

As mentioned above, contamination may disturb the proper functioning of an entire ecosystem. Both bioaccumulation and biomagnification are often observed in the trophic chain. Bioaccumulation is body's ability to bind poisonous compounds in cells or tissues. It is revealed in an accumulation of harmful substances in certain parts of an organism. With longer exposure time, a higher concentration of the compound is noted in the organism than in the polluted environment. The second issue, biomagnification, is more general and concerns more organisms at different trophic levels. It consists of a chemical substance accumulating and increasing in concentration while moving up through the trophic chain. This phenomenon is extremely dangerous and difficult to observe as well as to neutralize, due to its duration and the fact that the lowest levels of the trophic chain may exhibit a relatively low contaminant concentration in tissues or organisms, and this concentration can be low in an environment, as well (Mendrycka et al., 2013; Podsiadło and Krzyśko-Łupicka, 2013).

Along with the development of civilization, the need to effectively clean up the environment from harmful substances has increased. There are several methods for removing petroleum and its byproducts from soil. These techniques can be broadly divided into two groups, namely, physicochemical and biological. The first one includes the following processes: petroleum filtration, the removal of oily soil from a given area, and thermal deoiling of soil and waste. The latter, biological methods, are based on the metabolic activities of microorganisms (bacteria and fungi) to degrade hydrocarbons into non-toxic (CO_2 and H_2O in aerobic conditions) or more easily degradable substances. Phytoremediation is also grouped into this category. Bioremediation, including phytoremediation, seems to be much cheaper, more effective, and above all, more environmentally friendly than the previously mentioned approach. In addition, this solution is also not very invasive, yet relatively quick and economically viable (Henry et al., 2013). The biology-based strategy is currently gaining more attention in the treatment of contaminated areas. However, it requires a better understanding of 1) the mutual dependencies between the occurrence of different groups of organisms in a certain polluted habitat, 2) changes in the metabolic balance, and 3) the increasing biodegradability of a given hazardous substance. Taking all of this into consideration, there is a growing interest in using living organisms as effective degraders of chemical compounds, including hydrocarbons (Jaworska, 2012; Shahsavari et al., 2017).

Among all the available biological approaches, phytoremediation processes and phytoremediation combined with specially selected microorganisms seem to be good and effective alternatives (Bisht et al., 2014; Borymski and Piotrowska-Seget, 2014; Fester et al., 2014; Kluk and Steliga, 2016; Feng et al., 2017). Phytoremediation is based on a plant's ability to actively absorb and metabolize chemical mixtures containing substances with toxic effects on the environment and its components (Henry et al., 2013). This technique is mainly used to remediate brownfield sites, e.g., those contaminated with crude oil, petroleum products, or heavy metals and to limit and control the pollution of transportation routes (Grobelak et al., 2010; Traczewska, 2011; Glick, 2015). In the first case, mainly annual plants are used, whereas perennial plants (specifically, broadleaf trees) are applied in the second case.

Some plant species have individual and specific features that enable them absorb and transform compounds commonly considered as harmful for the environment. These organisms are able to actively determine and induce the natural processes occurring in the ecosystem (physicochemical and biological processes). Consequently, it allows for the proper conduction of metabolic processes for their cells, and thus their normal life cycles are maintained (Gałązka and Gałązka, 2016; Kosnar et al., 2018). Moreover, the organisms used in these phytoremediation techniques also have mechanisms that enable pollutant biotransformation/accumulation in tissues. This leads to lower concentrations of toxic substances in the environment (Marecik et al., 2006; Barabasz et al., 2008). To select appropriate cooperating species, it is very important to consider several aspects, such as soil type and geophysical and atmospheric conditions, in a given habitat.

Phytoremediation – its role in environment: processes, advantages, and limitations

Plants selected for phytoremediation treatment should be characterized by rapid and high-biomass growth; resistance to changing or unfavorable environmental conditions; a tolerance to the presence of pollutants (even in very high concentrations); a high absorption rate of toxic substances; resistance to several types of contaminants simultaneously (Marecik et al., 2006; Traczewska, 2011; Karczewska, 2012); and increased resistance to diseases and pests. Some species demonstrate an ability to incorporate toxic substances into the structure of their own cells. This is a natural adaptation to survive and exist in an unfavorable habitat (Baker and Brooks, 1989; Errington et al., 2018). Particular chemical compounds, such as enzymes secreted in the root system, can neutralize and inactivate certain contaminants. This results in their further migration through the plant's body and the environment being limited (Ali et al., 2013, 2014, Moubasher et al., 2015).

Phytoremediation can be generally divided into five categories, according to the type of internal activity of the plants:

a) **Phytostabilization** is a process in which soil reclamation is used to retain contaminants in the roots of selected plant species – hyperaccumulators or others characterized by an appropriate reaction to a given pollutant. This process takes place through adsorption on the root surface, absorption to the root interior, and precipitation in the root zone. In addition, the root system itself immobilizes the soil complex and pollutants, preventing accelerated erosion. Thanks to that, the contaminant is not transferred to deeper layers of the soil profile (Henry et al., 2013).

a) **Phytodegradation (Phytotransformation)** is a decomposition and biotransformation process of soil-accumulated pollutants thanks to the metabolic activity of plants and microorganisms. This is a phenomenon that occurs inside the plant, after the pollutants are collected by the roots, as well as outside the plant, e.g., due to enzymes released into the rhizosphere, which stimulate the development of microorganisms responsible for the degradation/detoxification of specific compounds. Different microbial groups are capable of utilizing various substances more effectively. The products of transformation may be used to build new plant tissues, evaporated by the stomata, or decomposed into simple inorganic compounds. Phytodegradation has been successfully applied to remediate soils contaminated with hydrocarbons, their chlorinated derivatives, and herbicides. Various species of poplar and willow are mainly used in this kind of treatment (Wang et al., 2008; Favas et al., 2014).

b) **Phytoevaporation (Phytovolatilization)** is a method consisting of removing contaminants from the water or soil and then transpiring pollutants or their modified (volatile) forms through the stomata into the atmosphere. Phytovolatilization occurs when growing plants take contaminants from the water or soil, and some of these pollutants transpire through their stomata. The method has also been applied in the removal of volatile contaminants, such as chloroorganic solvents and volatile organic compounds. Among the inorganic substances, phytovolatilization is useful in eliminating some heavy metals from various environments, e.g., water and soils (Soleimani et al., 2010; Podsiadło and Krzyśko-Łupicka, 2013).

c) **Phytoextraction** is based on the ability of higher plants to absorb pollutants through their root systems and subsequently translocate them to aboveground organs. Therefore, hazardous substances can be removed from the contaminated matrix together with the resulting biomass. Phytoextraction is mainly used in the treatment of soils and bottom sediments contaminated with heavy metals, radioactive elements, petroleum products, and other organic compounds. This process consists of three elementary steps: immobilizing the compound in the soil, removing the immobilized substance through the root system, and transporting it to the aboveground parts of the plant (Karczewska, 2012; Henry et al., 2013).

d) **Phytostimulation** is a process in which plants enhance the activity of microorganisms which are capable of transforming pollutants into more easily accessible and degradable forms. These products may then be used by other living organisms (e.g., plants). Microbial activity can be stimulated in several ways, i.e., a change of oxygen regime, a change of water–air relations in the soil complex, or by root-exuded substances (enzymes, sugars, etc.; Marecik et al., 2006).

A schematic representation of individual phytoremediation methods is shown in Figure 1.

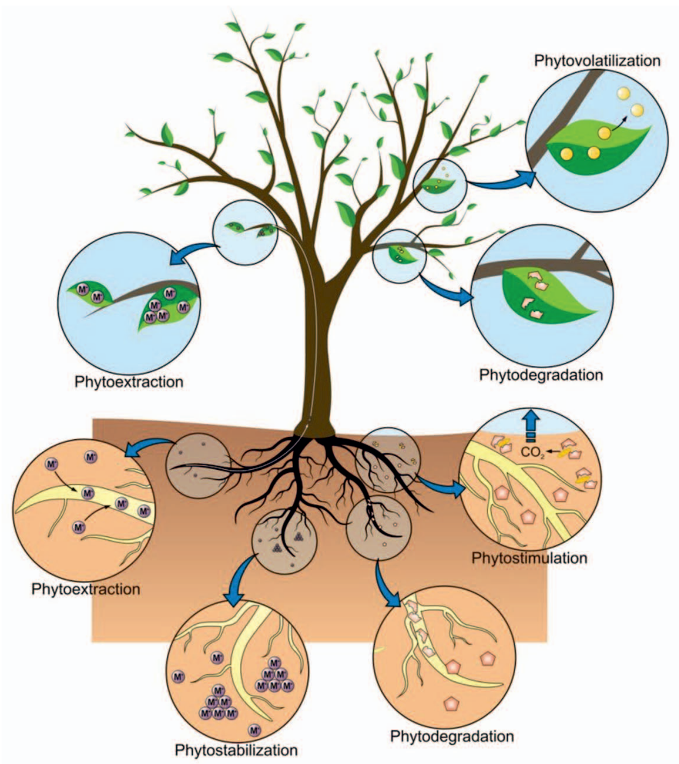

Fig. 1. A diagram of phytoremediation methods used to remove pollutants from the natural environment (based on Favas et al., 2014)

Rys. 1. Schemat metod fitoremediacyjnych stosowanych do usuwania zanieczyszczeń ze środowiska naturalnego (na podstawie: Favas et al., 2014)

The effectiveness of phytoremediation depends on various factors, namely, the plant species, the microorganisms used, the type of pollution, and the environmental conditions (Fig. 2). Among them, the type of plant is most crucial for the success of phytoremediation. Even within one species, pollutant removal may have a differentiated efficiency, and final concentration within the soil structure. Among plants, many grass and legume species are often chosen for these purposes (Baker and Brooks, 1989). Moreover, some of these organisms are hyperaccumulators. The potential of some grass and tree species has been recognized and appreciated. Due to their relatively large biomass, they are able to uptake and neutralize petroleum compounds and heavy metals in their cells in amounts comparable to those accumulated by hyperaccumulators (Burken et al., 2011). The soil type and its granulometric composition, water content, and water–air balance, as well as microbial diversity, are also important elements which may significantly support and strengthen the transformation processes. The decomposition time of a given product depends on its chemical composition and the bioavailability of its components. In the case of petroleum contamination, various compounds (hydrocarbons and heavy metals) are absorbed and/or transformed at different rates. The degree of degradation and concentration of the substances are equally important. In addition, the presence of a diverse and rich microbiome will contribute to the pollutant's transformation into more readily available products for plants and other organisms.

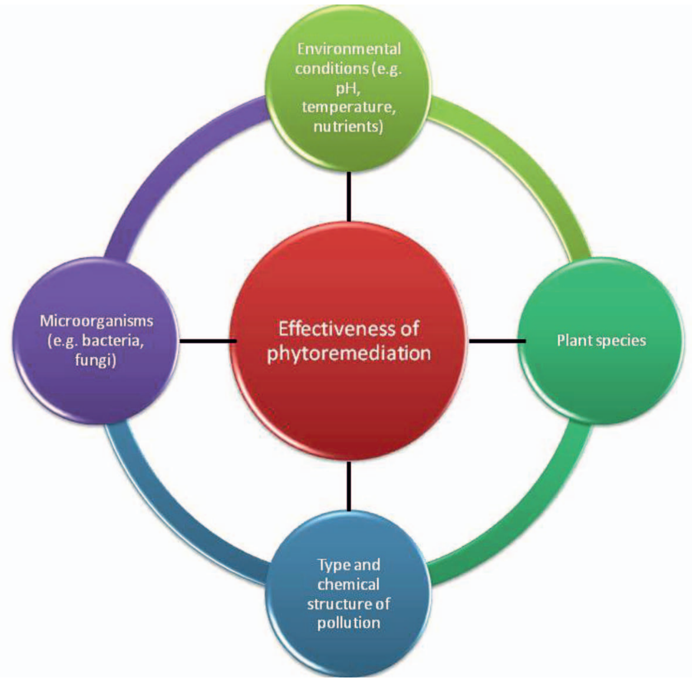

Fig. 2. A diagram showing the interdependence and influence of various factors on phytoremediation's effectiveness

Rys. 2. Schematyczne przedstawienie współzależności i wpływu różnych czynników na efektywność fitoremediacji

In implementing phytoremediation to cleanup contaminated sites, one needs to check and consider several issues. Firstly, how will the application affect the course of local ecological processes? Secondly, it is important to choose appropriate plants which demonstrate the required properties. The occurrence of biomagnification should also be taken into account. That is, the plant should not serve as sustenance for herbivorous animals and insects which provide a food base for higher-level consumers, including humans.

With the use of phytoremediation, it is possible to effectively cleanup a wide range of water, soil, and transitional environments (forming a connecting zone) from various pollutants. It is a very attractive and competitive method, in comparison to the conventional ones (mechanical or chemical). Phytoremediation can be considered an independent process as well as a complementary element of the most commonly used remedial approaches. The simultaneous activities of plants and microorganisms cause accelerated hydrocarbon degradation and heavy metal neutralization. Moreover, this cooperation seems to be the most potent and cost-effective solution.

Research confirms that specific groups of plant organisms can favorably influence the intensification of remediation processes in the case of petroleum-contaminated soils as well. It is recommended to use selected genera in areas exposed to outflows or oil spills, e.g., around oil wells, places of uncontrolled or accidental leaks, gas stations, or storage tanks. It has been proven that some plants are more resistant to pollutants, and can actively participate in removal of various hydrocarbons, e.g., polycyclic aromatic hydrocarbons (Feng et al., 2017).

Taking into account their action on contaminants, organisms can be divided into two different groups, namely, hyperaccumulators and degraders (Marecik et al., 2006; Weiner, 2012). Hyperaccumulators are characterized by above-average resistance to a specific toxic substance and its accumulation in the tissues. This kind of plant effectively uptakes higher concentrations (up to a 50–500-fold increase) of a compound than the average content absorbed by organisms growing in a pristine habitat. Despite such accumulation, hyperaccumulators do not show signs of adverse effects, to a certain extent. Depending on the plant type, the pollutant's availability, and its form, the final concentration in the plant's cells and tissues will vary according to the stress factor (Baker and Brooks, 1989; Van der Ent et al., 2013).

There are several probable theories which explain the development of this ability. The first one postulates that plant survival in a contaminated environment occurs by neutralizing toxic compounds via metabolic and physiological processes, while the other theory states that the absorbed elements may possibly be used as a kind of defense agent against individuals competing for habitats. On the other hand, accumulation of some compounds in the tissues may protect the plant against herbivores and pathogens. There is also a theory which suggests that the hyperaccumulation is completely accidental and it is revealed with occurrence of pollution (Van der Ent et al., 2013; Krzciuk, 2015).

A good hyperaccumulating organism should be characterized by features such as fast and high-biomass growth; a well-developed root system; the ability to accumulate and tolerate a significant amount of certain compounds and to actively transport them from the roots to other tissues or organs; and resistance to the presence of pests and pathogens. The hyperaccumulator should also have an effective herbivore deterrent system in order to prevent the migration of toxic substances to higher trophic levels. Additionally, the plant should be easy to grow and harvest when it is being considered for use as a potential agent in the remediation of contaminated areas (Ali et al., 2013). Pollutant removal is a huge challenge due to the multitude of physicochemical, atmospheric, and biological factors which constantly affect a living organism. Thus, the use of hyperaccumulators is an increasingly appreciated alternative to conventional remediation methods (Jaworska, 2012; Weiner, 2012).

Using metabolic and physiological processes, a degrader secretes enzymes and other biomolecules which allow it to mineralize or degrade a contaminant into non-toxic or less toxic substances. They are often simpler and more bioavailable to other organisms living in the contaminated ecosystem. In this way, the toxicity of pollution may be diminished. Among these organisms, there is a group which is capable of metabolizing hydrocarbons. Hydrocarbon degraders mainly include microorganisms, especially some bacteria and fungi, and they are able to use such compounds as a source of carbon and energy. Some of the biotransformed compounds (byproducts) which are not further utilized by the degrader may in turn be used by other organisms inhabiting the same niches. The use of appropriate microorganisms is very beneficial for the ecosystem. Under optimal conditions, microbes multiply rapidly, creating a suitable and effective tool for removing environmental pollution. To be introduced directly into the habitat, potential degraders:

- cannot be pathogens of native flora and fauna;
- should not produce toxins; and
- should not be competitive with indigenous organisms (Gerhardt et al., 2009; Weiner, 2012).

Contaminant removal enhanced by plant organisms is widely applicable. Namely, it can be used for the remediation of soils, sediments, and surface and groundwaters. The undoubted advantages of phytoremediation are a) it is possible to apply it "on-site"; b) no additional chemical treatments are needed; c) the chances of secondary pollution are limited; and d) the substrate is protected against erosive processes and variable weather conditions by a compact root system. An additional benefit is that the plants are esthetically attractive. The downsides of these techniques are primarily the relatively long duration of the process and its efficacy's dependence on many environmental factors (e.g., the physicochemical properties of soil and seasonal temperature fluctuation). These factors may lead to differentiation of the final results. Another aspect is that the zone of phytoremediation's effectiveness is mainly limited by the depth of root growth. Therefore, contaminants below the root zone will not be retained and neutralized (Fatima et al., 2015).

An examination of various groups of organisms provides an opportunity to estimate and predict the possible flow of substances in the trophic chain, especially on higher levels such as non-human primates and humans. Analyses of the status of individual elements allow for a comprehensive assessment of the quality of the environment being studied and the choice of the best remedial strategies. Biological methods, including phytoremediation technologies, are currently considered as one of the most important trends in the renewal of natural resources. They are effective and very economically profitable techniques. As above mentioned, they seem to be non-invasive and ecosystem-friendly compared to traditional solutions. Those are often very unfavorable and cause irreversible, often drastic changes in soil structure and properties.

Generally, phytoremediation has gained public acceptance. It has also been successfully applied in cases of minor pollution, ecological disasters, and the release of organic compounds – mainly oil-derived substances – into the environment (Shahsavari et al., 2017; Baoune et al., 2018). More and more, biological methods are applicable in the clean-up of hydrocarbon-contaminated environments. The most efficient and cost-effective approach seems to be the simultaneous use of appropriate plants and cooperating microorganisms which support the degradation, neutralization, and removal of toxic substances from a specific habitat.

Cooperation between plants and microorganisms

Scientific sources say that most plants on our planet function and can effectively defend or neutralize pollution thanks to the presence of microorganisms. The phenomenon by which plants' roots are colonized by mycorrhizal fungi has been observed in nearly 95% of the studied plant organisms (Duponnois and Garbaye, 1991). More attention is paid to the synergy between plants and other organisms, primarily bacteria and fungi, in remediation processes. Microorganisms inhabiting the plant's root zone may significantly contribute to the phytoremediation success. Rhizosphere bacteria can stimulate plant growth by supplying minerals and synthesizing phytohormones. Moreover, these microorganisms can protect plants against the negative effects of organic pollutants and heavy metals, and against the action of phytopathogens (Gerhardt et al., 2009; Hou et al., 2015; Moubasher et al., 2015). Examples of cooperating organisms and the substances which are degraded by them are shown in Table 1.

Table 1. Examples of microorganisms and host plants used in the phytoremediation of soils contaminated by petroleum hydrocarbons
Tabela 1. Przykłady mikroorganizmów i roślin współdziałających w fitoremediacji gleb zanieczyszczonych węglowodorami ropopochodnymi

Petroleum hydrocarbons	Microorganism	Host plant [reference]
PAHs	*Pseudomonas* spp., *Neotyphodium coenophialum*, *Neotyphodium uncinatum*	*Halimione portulacoides* [Oliveira et al., 2014], *Festuca arundinacea* (tall fescue) [Soleimani et al., 2010], *Festuca pratensis* (meadow fescue) [Soleimani et al., 2010]
PAHs, alkanes	*Pseudomonas* sp., *Microbacterium* sp., *Rhodococcus* sp.	*Lolium parenne* L. (ryegrass) [Kukla et al., 2014]
Phenanthrene	*Pseudomonas putida*, *Massilia* sp., *Paenibacillus* sp.	Willow [Khan et al. 2014], *Alopecurus aequalis 'SOBOL'* (shortawn foxtail 'SOBOL') [Liu et al., 2014] *Plantago asiatica* [Zhu et al., 2016]
Crude oil	*Acinetobacter* sp.	*Brachiaria mutica* [Fatima et al., 2015]
Toluene	*Burkholderia cepacia*	*Populus trichocarpa* (California poplar) [Taghavi et al., 2005]

PAHs – polycyclic aromatic hydrocarbons

Since microorganisms play an essential role in phytoremediation processes, their identification is an important and valuable aspect. Bacteria can use selective mechanisms that affect the interaction between the environment and living organisms (Rigamonte et al., 2010; Feng et al., 2017). Such an influence may lead to a positive or negative response between individual elements. These microorganisms can perform several basic functions, i.e., supporting the roots' receptivity to mycobiont, recognizing the correct fungus, strengthening the growth of the fungal organism, and modifying the soil properties in the root zone and in the germination of fungal propagules (Frey-Klett et al., 2007).

There are two main microbial groups supporting plant activity, namely, mycorrhiza-helper bacteria (MHB) and plant-growth-promoting rhizobacteria (PGPR) (Hou et al., 2015). MHB seem to develop an increased specificity towards mycobionta. However, they do not show or only slightly show specificity towards the host plant. In general, mycorrhiza-helper bacteria can be divided into two main categories. The first group consists of organisms that stimulate the process of mycorrhiza formation, while the second one includes those bacteria which positively affect the course, functioning, and efficiency of a plant–mycobiont system. Different types of microorganisms will belong to each group depending on the environment, the host plant, and the symbiotic fungus. Among MHB, organisms belonging to the *Pseudomonas* genus are the most abundant. Recent data show that these symbiotic relationships have been occurring for at least 50 million years. This fact indicates that these relationships should be beneficial, since they were created and maintained by evolutionary processes (Lepage et al., 1997; Lynch, 1990). Many MHB are also classified as PGPR (Shiley et al., 2007). In the environment, these microorganisms are used by plants to reduce ecological stress, and they play an important role in the degradation of petroleum products (Rigamonte et al., 2010). Both laboratory and field results demonstrated increased phytoremediation effectiveness when plants characterized by an appropriate tolerance to unfavorable conditions and plant-interacting microorganisms (including PGPR) were used (Hou et al., 2015). Such mutual cooperation of organisms definitely brings the best effects for the remediation of hydrocarbon-contaminated soils. Depending on the soil type and composition and the type of contamination, the response of organisms and the final yield of substance degradation will vary. The results of tests carried out under different conditions have led to the conclusion that the use of plant and microorganism symbiosis is a very effective clean-up method (Hou et al., 2015; Feng et al., 2017, Kong et al., 2018). It is also important to note that depending on the metabolic processes, substrate, and ecological specialization, different microbial groups will be able to degrade the relevant compounds from oil (Kong et al., 2018). Pollutants which are generally classified as the most difficult compounds to degrade – mainly polycyclic aromatic hydrocarbons (PAHs) – can be effectively removed through the use of phytoremediation enhanced with microorganisms (Peng et al., 2009; Guarino et al., 2017).

Investigating and assessing the efficacy of cooperation between specific genera of organisms requires accurate knowledge. To apply an appropriate treatment, it is necessary to identify species capable of degrading a given compound under specific conditions. In an ecosystem, all living organisms constantly interact with each other, causing diverse biological responses or determining the occurrence or disappearance of some property. Therefore, a contaminated soil complex, like other habitats, should be considered as a whole. Both abiotic and biotic factors should be taken into account to best protect the environment and to restore the original conditions. Thus, further observations and studies are important and necessary to assess the effectiveness and usefulness of both the phytoremediation process and phytoremediation supported by microorganisms. The identification of bacteria and fungi which can cooperate with specific plant species is an essential aspect. As mentioned above, this will allow for the selection of appropriate remedial agents to maximize the effectiveness of the removal process.

Before phytoremediation technologies can be applied in a given contaminated terrain, many key issues are crucial to consider. One of the most essential things is how these processes will affect the course of local ecological relationships. After completing the clean-up, a big problem is collecting the biomass and subsequently protecting against the compounds which were removed from re-entering the environment. Unfortunately, it is not possible to regard such vegetation as a source of food (i.e., for animals) due to the significant amount of accumulated contamination. Currently, the main and practically the only method of effective removal is transforming the resulting biomass into energy and possibly recovering some of the elements (i.e., metals). Combustion, fermentation, and thermochemical and gasification processes are used for this purpose. The final products, such ashes and sludges, must be stored in properly secured places or can be transported back to the plant which ordered the phytoremediation treatment. The main advantages of the former solution is a reduction in the biomass volume and amounts of toxic substances that could potentially return to the environment during storage. With the use of phytoremediation, it is possible to effectively decontaminate a wide range of habitats, e.g., water, soil, air, and transitional environments (combining the properties of more than one) from a wide range of pollutants. It is a very attractive and competitive approach in comparison to traditional (e.g., mechanical or chemical) land treatment forms. It can be used as an independent process or as a complementary element of frequently applied remediation methods. Moreover, phytoremediation is a good ecological solution.

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