บทความวิชาการ

การฟื้นฟูดินปนเปื้อนร่วมกันระหว่างสารประกอบปิโตรเลียมไฮโดรคาร์บอน และโลหะหนักด้วยพืชภายใต้ภาวะความเค็ม

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บทคัดย่อ

การฟื้นฟูดินปนเปื้อนร่วมกันระหว่างโลหะหนักและสารประกอบปิโตรเลียมไฮโดรคาร์บอนภายใต้ภาวะความเค็มเป็นสิ่งจำเป็นต่อการดูแลรักษาและพัฒนาระบบนิเวศดินให้ยั่งยืน ในปัจจุบันการฟื้นฟูด้วยพืชซึ่งเป็นเทคนิคที่สะอาดและมีความปลอดภัยต่อสิ่งแวดล้อมได้ถูกนำมาใช้สำหรับแก้ไขปัญหาการปนเปื้อนร่วมกันของสารมลพิษ โดยพืชที่ใช้ฟื้นฟูสภาพดินปนเปื้อนภายใต้ภาวะความเค็มนั้นจะต้องมีคุณสมบัติที่สำคัญในการทนต่อความเค็มและมีความสามารถในการกำจัดสารปนเปื้อนได้ดี แม้ว่าพืชฮาโลไฟต์จะมีความเหมาะสมที่สุดสำหรับกระบวนการฟื้นฟูดินปนเปื้อน แต่การใช้พืชฮาโลไฟต์บางชนิดก็มีข้อจำกัดอยู่พอสมควร ความสามารถในการกำจัดการปนเปื้อนร่วมกันของสารมลพิษก็เป็นปัจจัยจำกัดที่มีผลต่อประสิทธิภาพสำหรับกระบวนการฟื้นฟูด้วยพืชอีกด้วย ทั้งจากพิษของโลหะหนักต่อประสิทธิภาพการย่อยสลายทางชีวภาพของสารประกอบปิโตรเลียมไฮโดรคาร์บอนและความเป็นพิษของสารประกอบปิโตรเลียมไฮโดรคาร์บอนต่อปริมาณการสะสมโลหะหนักในพืช ยิ่งไปกว่านั้นความเค็มของดินก็เป็นสาเหตุให้มีการสะสมโพรลีนเพิ่มมากขึ้น ขณะที่การเติบโตและปริมาณคลอโรฟิลล์ของพืชลดลง ซึ่งความเค็มยังมีบทบาทที่สำคัญต่อการย่อยสลายสารประกอบปิโตรเลียมไฮโดรคาร์บอนโดยจุลินทรีย์และการเคลื่อนที่ของโลหะต่อพืช ดังนั้นการคัดเลือกชนิดพันธุ์พืชที่มีประสิทธิภาพในการกำจัดสารปนเปื้อนร่วมกันระหว่างโลหะหนักและปิโตรเลียมไฮโดรคาร์บอนนั้นถือว่าเป็นสิ่งที่จำเป็น

คำสำคัญ: การฟื้นฟูด้วยพืช การปนเปื้อนร่วมกันของสารมลพิษ ความเค็ม

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Phytoremediation of Heavy Metal and Total Petroleum Hydrocarbon Co-contaminated Soil under Salinity Condition

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ABSTRACT

Remediation of heavy metal and petroleum hydrocarbons co-contaminated soil under salinity is essential to sustainable development and maintenance of soil ecosystems. Recently, an environmentally safe and clean technique known as phytoremediation has been introduced to address the co-contamination problem. Plant properties important for phytoremediation of contaminated soil under salinity condition are ability to tolerate soil and remove contaminants. Although halophytes are appropriate for phytoremediation of contaminated soil, the application of some halophytes is rather limited. Co-contamination of these pollutants limits the efficiency of phytoremediation both from heavy metal toxicity to potential biodegradation of total petroleum hydrocarbons (TPHs) and TPHs toxicity to heavy metal accumulation in plants. Moreover, the presence of salt caused a marked reduction in plant production, the total chlorophyll content in plant tissues, and high accumulation of proline. Salinity plays an important role in the degradation of TPHs by microorganisms and metal mobility to plants. Therefore, heavy metal and TPHs removal by vegetation can be greatly enhanced by the judicious selection of plant species.

Keywords: Phytoremediation, Co-contaminated soil, Salinity

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Introduction

Total petroleum hydrocarbons (TPHs) are a term used to describe a broad family of several hundred chemical compounds that originally come from crude oil. TPH compounds are the primary component in various petroleum products; therefore, TPHs are mostly released as a result of petroleum product spills either on land or water [1]. The physical and chemical properties of some TPH compounds and petroleum products containing TPHs are well defined and can be used to estimate the fate of TPH transport fractions following release to the environment [2]. More information on the production volumes for various petroleum products, environmental releases, and disposal would aid in assessing the potential for human exposure to TPHs from accidental or intentional releases [1]. Transition from TPHs to metals are often characterized and distinguished from non-metals by their physical properties, ability to conduct heat, and an electrical resistance that is directly proportional to temperature, malleability, ductility and even luster [3]. However, the physical properties are lost after the metal has been chemically transformed into a chemical compound that can be taken up by plants [4].

Co-contamination of TPHs and metals is a common feature in nature [5]. Approximately 40% of hazardous waste sites on the US EPA's national priority list (NPL) are co-contaminated with both organic (TPHs, PAHs and PCBs) and heavy metal (As, Cd, Cr, Cu, Pb, Hg, Ni and Zn) pollutants [6]. Soils polluted with heavy metals and organic pollutants pose a health hazard to humans, as well as plants and animals [7]. TPHs are considered to be one of the major organic pollutants, particularly in the present time [8]. Furthermore, they are also known as toxic to many living organisms [9].

Saline soil is a critical problem for soil in agriculture. However, some of the saline soils are polluted with heavy metals and gasoline. This because is soil pollution may come from many oil companies storing leaded gasoline underground and near the sea. Storage tanks leaked a large amount of heavy metals and petroleum fuel, thus contributing to the contaminated saline soil. When pollutants (organic and inorganic) are mixed or combined, phytoremediation efficiency may be impaired as contaminants may interact among themselves as well as with plants and the rhizosphere [10, 11]. Estimated remediation may vary due to different conditions of environment (physical soil properties and salinity), including the type of pollution which is considered a key factor limiting the efficiency of phytoremediation under field conditions [12]. This review focuses on phytoremediation under saline conditions and the possible ways to achieve the remediation of co-contaminated soil, with the understanding of plant-microbe interactions for the remediation of heavy metals and TPHs in salt-affected soils with a potential for bioremediation.

Heavy metal and TPHs remediation by plant

For a long time, phytoremediation of hydrocarbons and metal contaminated soils, in which plants are used to remove, contain or detoxify environmental contaminants [13], offers a better and more environmentally friendly alternative than conventional soil remediation alternatives. Plants can absorb a minute quantity of TPHs from the soil and translocate them into different parts [14]. TPHs uptake and accumulation in the root and shoot of plants vegetated in contaminated soil are associated with contaminant concentration in the soil [15]. Once TPHs move into the plant, they may have multiple fates; some hydrocarbon compounds can be sequestered in the root tissue, some can be transported into shoots and then leaves, where they can be stored in the vacuole or volatilized into the atmosphere [14]. In contrast, in the phytoremediation of heavy metals, the metals may only be mobilized or immobilized. Unlike hydrocarbons they cannot be degraded to less innocuous components. Phytoextraction and phytostabilization of heavy metals are major principles for cleaning of metal contaminated soil [16]. Phytoextraction is done by extraction of heavy metals, translocation to aboveground storage tissues, sequestration of elements in the root system which prevent the spread back into soil/groundwater, and, finally, conversion into less toxic chemicals [17]. However, the presence of heavy metals usually adversely affects plant health. Cd and Pb in plants have been shown to interfere with and inhibit various physiological processes [6].

Heavy metal and TPHs remediation by plants is affected by many factors, such as plant species variations, stage of plant growths, accumulation and translocation of contaminants. It is generally believed that for the cleanup of soil contaminated with TPHs and heavy metal, plants should have the ability to tolerate high concentrations of these pollutants and possess an extensive root system. On-site phytoremediation of TPHs and heavy metals can be enhanced by employing a combination of common agronomic practices (e.g. fertilizer application and irrigation). This is because available nutrient reserves in the soil can be quickly depleted as the microbial community begins to degrade the contaminants [18]. Therefore, fertilizer applications may enhance the degradation of petroleum hydrocarbons in soil by reducing competition for limited nutrients. There are only a few studies, however, on the phytoremediation of soil co-contaminated with TPHs and metals. Plant species showing potentials for remediation of co-contaminated soil include crop plants such as corn *(Zea mays),* ornamental plants *(Tagetes patula),* mangroves *(Rhizophora mangle),* alpine pennycress *(Thlaspi caerulescens)* and an invasive weed *(Chromolaena odorata)* [5, 19-22]. Other studies have been focused on the use of grasses for the remediation of TPHs and heavy metals due to their ability to tolerate TPHs or both, an extensive root system, a large root surface area and deeper root penetration into the soil [5, 23-27]

Heavy metal and TPHs remediation by plant-bacteria interactions

Although phytoremediation provides an ecologically and economically attractive technique for contaminated soil remediation, one of the major limitations of using this approach is the fact that many plants species are sensitive to contaminants [28]. To overcome this problem, the combination of bioaugmentation and phytoremediation is required for remediation, resulting in microbe-assisted phytoremediation, which could solve some of the problems encountered during the application of both individual techniques [29]. Moreover, the application of microorganisms in phytoremediation helps to improve plant growth and survival rate [30]. It is assumed that, micro-organisms degrade or transform contaminants through a variety of mechanisms. Contaminants are transformed by living organisms through reactions that take place as a part of their metabolic processes [31]. Microorganisms are less suitable for the bioremediation of heavy metals than they are for organic contaminants, since metals are not biodegradable, and microorganisms can only change the speciation of metal contaminants [32]. Nevertheless, rhizospheric bacteria were reported to be involved in metal uptake into plant tissue. Other studies evidenced that the organic compounds released by plant roots act as signaling agents and perform an important role in plant-bacteria interactions for the removal of contaminants [33, 34]. There are various reasons for plants to promote the degradation of hydrocarbons by immobilizing and removing them, and then by enhancing microbial degradation [35]. Simultaneously, plants can remove heavy metals from the soil by incorporating them into their tissues [36].

The main mechanism involved in the remediation of TPHs is believed to be biodegradation in the rhizosphere or rhizodegradation caused by the activities of microorganisms present at the rhizosphere of the plant [37]. Plants and their associated bacteria interact with each other, while the plant supplies the bacteria with special carbon sources (carbohydrates, organic acids, amino acids) that stimulate the bacteria to degrade TPHs [38]. Furthermore, these bacteria can enhance the solubility of inorganic phosphates by synthesizing organic acids, some can degrade organic compounds containing phosphates by releasing enzymes phosphatases, which are preferably absorbed by plants [39]. Several researchers reported that the growth of some plants of requisite root exudates into soil with hydrocarbons due to increased bioavailability of TPHs for enhanced biodegradation by providing an easily degradable energy source [40, 41]. This synergistic action of plants and inoculated bacteria, resulted in more efficient rhizodegradation of hydrocarbons when compared to microbial remediation and phytoremediation (without bacterial inoculation) [42]. Plant-associated bacteria, in relation to heavy metals and TPHs co-contamination, have been mostly addressed in the context of phytoremediation, and the efficiency of remediation can vary due to species-specific interaction of rhizobacteria. Therefore, knowledge of the abilities of different plant species or tissues to absorb and transport metals under different conditions may provide important insights into choosing appropriate plants for phytoremediation of the polluted regions.

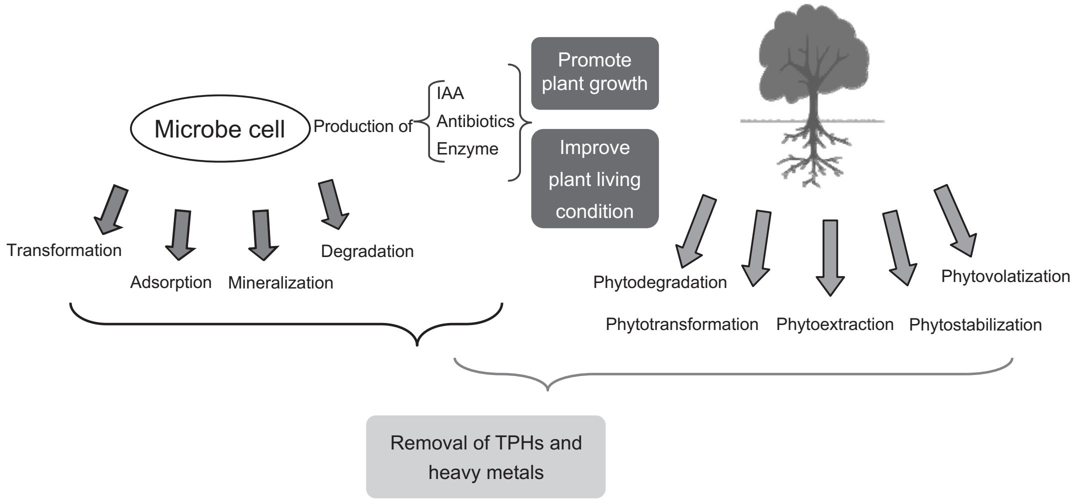

Figure 1 Biodegradation mechanisms involved in the plant-associated bacteria for the removal of TPHs and heavy metals, modified from Teng *et al.* [43]

For efficient hydrocarbon remediation, it is of primary importance that the inoculated hydrocarbon-degrading bacteria colonize the rhizosphere and plant interior in order to mediate their effects on plant growth and contaminant degradation [44]. In multi-contaminated soils, trace elements may modify root growth and thus affect root enhanced dissipation, or exert direct effects on microorganisms and thus affect pollutant degradation [45]. Lin *et al.* [46] reported that a high Cu level was unbeneficial to pyrene dissipation in corn, which may be due to a change in microbial community or activity, or modified root physiology under Cu stress. The presence of Cd may impede rhizodegradation of organic compounds [45]. Since the degradation of organic pollutants during phytoremediation depends extensively on the presence of suitable microbes and microbial activity, if metals negatively affect microbes and limit their activity, the success of phytoremediation may be severely compromised [47].

Effects of salinity and contaminants (heavy metals and TPHs) on plant growth

Primary salinity occurs when water-soluble salts accumulate in the soil to a level that impact on soil ecosystems, especially in agricultural soils where high salinity may be a result of irrigation practices and the application of chemical fertilizers. Saline soils go through many physical changes compared with normal and healthy soils. Salinized soils are observed to have low structural stability, infiltration rate, water-holding capacity, organic matter and biological activity [48]. Soluble salts are highly mobile and these salts are easily accumulated in plants [49]. However, adaptation or tolerance of plants to salinity stress involves complex physiological traits and biochemical mechanisms. A detailed description of adaptation to salinity is given in Fig. 2 [50]. In addition, saline soils are seen to possess high concentrations of potassium ions, chloride ions, and sulfate ions. The chemical characteristics of soil are adversely affected by salinity because it results in increased heavy metal ion concentrations. An increased salinity, which is a cation, may contribute to cation displacement of heavy metal stored in the sediment [51].

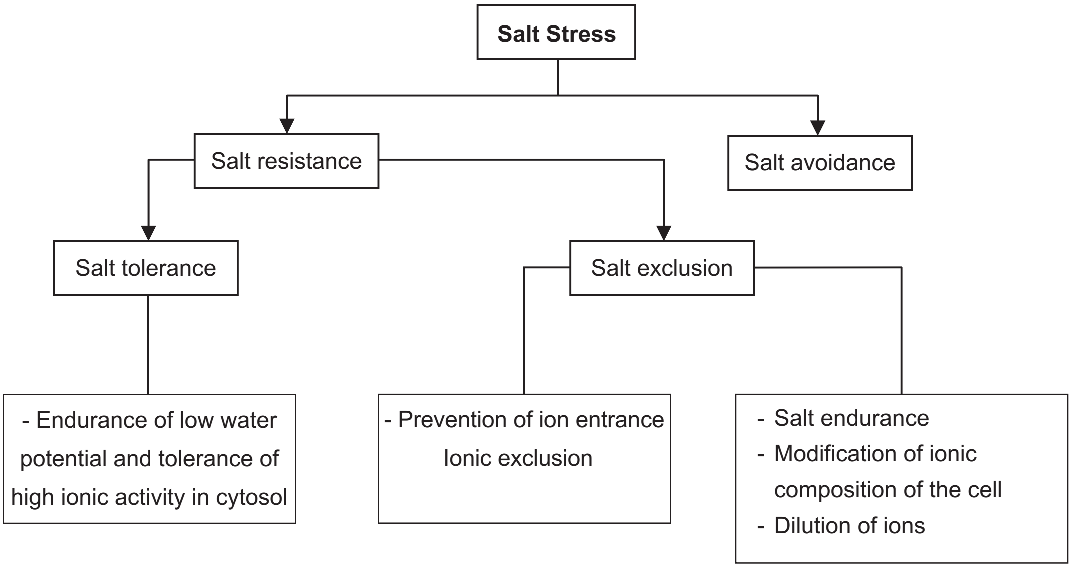

Figure 2 Modes of plant adaptation to salinity [50].

In saline soils, increased salinity results in a progressively smaller and more stressed microbial community, which is also seen to be the least efficient in terms of metabolism because salt enhances the degradation of organic matter [52]. Salinity reduces soil microbial biomass and microbial activity, which in turn changes the carbon dioxide fluxes. Moreover, soil salinity imposes ion toxicity, osmotic stress, nutrient (N, Ca, K, P, Fe and Zn) deficiency and oxidative stress on plants, and thus limits water uptake from the soil [53]. In addition, salt intrusion into the soil solution causes water deficits in leaves and inhibits plant growth and metabolism [54]. A 2015 study demonstrated that salt stress (7.79 ds/m) affected the biomass and shoot height of *C. odorata,* and it was less tolerant to salinity than to contaminants (780 mg/kg Pb, 27,000 mg/kg fuel oil) during 90 days of treatment [55]. Salinity reduces the ability of plants to take up water, and this quickly causes reduction in growth due to a decrease in the cell number and tissue expansion. Furthermore, the findings also demonstrate the negative effects of salinity on the level of proline accumulation in *C. odorata.* High salinity can cause osmotic stress in plants, and thus accumulation of osmoprotectants in the cytoplasm was an important mechanism to maintain the osmotic balance with the external medium and vacuole in plants [56]. It has been observed that many halophytic plants accumulate proline in response to salinity stress [56]. Accumulation of proline in response to heavy metals (Cu, Cd, Zn and Ni) and salinity has been described in several plant species and has been reported to indicate a general response to stress in plants [57, 58]. Rastgoo and Alemzadeh [59] reported that numerous distinct heavy metals (Cd, Co, Pb and Ag) increased the concentration of proline in *Aeluropus littoralis.*

Additionally, heavy metals and salinity often have deleterious effects on photosynthesis in plants. Several previous studies reported reductions in chlorophyll content in plants exposed to heavy metals, petroleum hydrocarbon, and high salinity [60-62]. However, the total chlorophyll content in the salt-tolerant plants, *Tamarix smyrnensis and Atriplex halimus* were unaffected by heavy metals (Cd, Pb) or salinity [63, 64]. It is clear that the effects of soil salinity on plant growth are complex, causing damage and inhibition to various plant physiological processes. Therefore, a selection of plant species that can withstand elevated levels of salinity and capable of producing adequate biomass under salt stress is one of the most important criteria for phytoremediation.

Effects of salinity on heavy metal accumulation and TPHs degradation

Many plant species able to accumulate a high quantity of salts are used as phytoremediators [65]. Several studies have reported that heavy metals induce in plants both a secondary water stress and an oxidative stress, the capability of halophytic plants to synthesize those organic compatible solutes may be involved in their ability to cope with heavy metals [66-68]. Furthermore, the tolerance of halophytes to salt stress is generally correlated with a more efficient antioxidant system, and thus, halophytes are expected to have better tolerance against heavy metals stress in comparison to salt-sensitive crop plants [69]. Likewise, halophytes can be exploited as a significant and major plant species bearing potential capability for the desalination and restoration of saline soils and phytoremediation as well. Agoramoorthy *et al.* [70] demonstrated that five halophytic species were efficient accumulators contributing to reduction of soil and water contamination by heavy metals in mangrove ecosystems. However, several halophytes species had rather limited uses. This is because halophytes show low biomass productions and a shallow root system, and the technology for their large-scale cultivation is not fully developed [71].

Plant abilities for phytoremediation are determined by ion uptake, root to shoot translocation of ion, intracellular sequestration, and chemical modification and stress resistance [72]. Clearly, plants in combination with rhizospheric bacteria have been shown to remediate soil co-contaminated with heavy metals and crude oil [21]. However, heavy metals and high salinity together decrease microbial populations and diversity, and affect microbial community composition [73]. On the other hand, several studies indicated that salinity increases metal mobility. The increasing salinities in soil can improve the mobilization of heavy metals due to competition with Ca^{2+}/Mg^{2+} in the saline soil, and promote the metals uptake by crops [74]. Furthermore, soil salinity may stress crops, activate the crop ion transport system and therefore promote the translocation of heavy metal ions into crops [75]. It has been reported that mustard *(Brassica sp.)* is observed to uptake and accumulate high concentration of heavy metals in saline environments [76, 77]. In addition, other studies have reported the positive effects of salinity on metals accumulation and uptake especially for Cd. Increasing salinity increased Cd uptake and accumulation in halophyte species *T. smyrnensis* and *A. halimus,* sunflower and sudangrass [63, 64, 78]. Similarly, salinity increased the mobility of heavy metals (Cd, Cu, Mn, Pb and Zn) in soils and sediments [79, 80]. This increase in metal mobility depends on the total amount of heavy metals present and the type of salt inducing the salinization [74]. Zhao *et al.* [81] also recommended an increase of salinity which promoted metal mobility which followed the order: Cd > Mn > Cu > Pb. This may be related to a higher bioavailability of the metal in soil due to increased Cd sorption in soil particles [82], and/or higher water uptake leading to a higher flux of metals into the plant [83]. According to the above, however, not only halophytes but also of plants in general are potentially ideal plants for remediation of heavy metals contaminated saline soils, and moreover of heavy metal uptake affected either negatively or positively, by salinity.

In addition to TPHs, several studies showed enhanced degradation of crude oil in polluted soil, without salinity, under the influence of plants. Relatively high and moderate percentage of TPH reductions were reported in *R. mangle* (87%), *C. odorata* (80%), *Cyperus odoratus* (78%), *C. laevigatus* (73%), *Mirabilis jalapa* (41.6-63.2%), ryegrass (52%) and bermuda grass (40%) [5, 22, 84-86]. There have been some reports whereby plants alone were used successfully for bioremediation of TPHs from contaminated soils [84, 87]. However, several previous studies show that TPH degradation rate decreased in the presence of salinity [88-90]. For the remediation of co-contaminants in highly saline soils, both plants and bacteria must be able to cope with multiple stress situations. It is well known that high soil salinity may be a naturally occurring problem in some contaminated areas, because salinity plays an important role in the degradation of TPHs by microorganisms [91]. The effect of salinity on the degradation rate include direct inhibition of metabolic activity may also cause by unfavourable high osmotic potential of the microbe's environment, and altered solubility or sorption of toxic or essential ions [92].

Under these considerations, the toxic effects of heavy metals, petroleum hydrocarbons, and high salinity on plants are manifested by an inhibition of photosynthesis, decreased production of photosynthetic pigments, reduction in growth, and even death. High salinity is believed to be responsible for inducing osmotic shock, disrupting cell membrane, and inhibiting various physiological processes, any of which could be lethal for bacteria. Hence, measurement of plant growth, soil microbial biomass and microbial activity can be used as an indication of the extent to which plant-associated bacteria are salt stressed during the remediation process.

Conclusions

Successful remediation of co-contaminated soil with high levels of salt is hard to achieve due to the fact that plant growth and germination, including plant root exudates lead to increase in the population of native soil microbes is inhibited by salinity. The decrease of plant biomass production on salt-impacted sites affected the phytoremediation efficiency because of direct toxicity and the fact that the plant biomass was simply insufficient for remediation. Therefore, salt tolerant plants that have deep and vigorous root growth, as well as sufficient above-ground biomass production are some of the basic criteria for the selection of plants for remediation of salt-impacted sites. Co-contamination of TPHs and heavy metal in saline soil is more complicated than single contaminated soil without salt. For the applications of phytoremediation for saline soil co-contaminated with TPHs and heavy metals, hyperaccumulating plant species are of interest as well as plants which produce high biomass, including tolerate salt and co-contaminants to a certain degree.

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