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Phytoremediation and Plant-Assisted Bioremediation in Soil and Treatment Wetlands: A Review

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Abstract: Phytoremediation is a technology that is based on the combined action of plants and their associated microbial communities to degrade, remove, transform, or immobilize toxic compounds located in soils, sediments, and more recently in polluted ground water and wastewater in treatment wetlands. Phytoremediation could be used to treat different types of contaminants including petroleum hydrocarbons, chlorinated solvents, pesticides, explosives, heavy metals and radionuclides in soil and water. The advantages of phytoremediation compared to conventional techniques are lower cost, low disruptiveness to the environment, public acceptance, and potentiality to remediate various pollutants. The use of plants in conjunction with plant associated bacteria (rhizosphere or endophytic) offers greater potential for bioremediation of organic compounds, and in some cases inorganic pollutants than using plants alone in bioremediation. The implementation of treatment wetlands for phytoremediation of wastewater or polluted water originating from various sources allows removing organic and inorganic pollutants from water in an environmentally friendly and economically feasible way.

Presently, different processes of phytoremediation in treatment wetlands are less studied compared to phytoremediation of polluted soils. Further research is needed to advance the understanding of the pollutant removal mechanisms in treatment wetlands with vegetation, and how based on this information to improve treatment wetland design and operational parameters to achieve more efficient treatment processes. This review covers basic processes of phytoremediation with special emphasis on rhizoremediation and plant-microbe interactions in plant–assisted biodegradation in soil and treatment wetlands.

Keywords: Rhizoremediation, phytoremediation, treatment wetlands.

INTRODUCTION

An increasingly industrialized global economy has led to dramatically elevated releases of anthropogenic chemicals into the environment over the last century and resulted in contamination of many areas on Earth. Contamination can be a result of improper chemical production (i.e. oil spills from drilling, explosives from manufacturing), transport (i.e. oil spills from tankers or pipelines), storage (i.e. chemicals from leaking storage tanks), usage (i.e. pesticides and fertilizers from agriculture, explosives from munitions firing) or disposal processes (i.e. explosives from demilitarization facilities).

Concurrently with increasing pollution levels, avid interest in developing strategies for remediation of environmental contaminants using physical, chemical and biological processes has emerged. As classic "suck and truck" strategies followed by off-site treatments are expensive, the *in situ* bioremediation processes like monitored natural attenuation (MNA), biostimulation, bio-augmentation and phytoremediation (incl. rhizoremediation) have become an attractive way to rehabilitate contaminated sites [1]. Besides the aforementioned methods, a variety of other remediation technologies are available for on-site remediation of polluted soils. Soil vapour extraction, landfarming, bioventing, thermal desorption, and biopiles have been used as real life applications for on-site soil clean-up [2]. However, no single technology is appropriate for all contaminant types and the variety of site-specific conditions which exist at different contaminated sites and often more than one remediation technology is needed to effectively address contaminated site problems [2]. Site conditions, contaminant type and source, source control measures, and the potential impact of the possible remedial measure determine the choice of a remediation strategy and technology. In recent decades, phytoremediation - a cost effective and environmentally friendly technology - has been used successfully for the remediation of soils contaminated with various pollutants. In addition, phytoremediation is increasingly used as a technological complement for treatment of polluted water in different types of treatment wetlands [3, 4]. However, compared to phytoremediation of

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the polluted soils the plant application in treatment wetlands for pollutant removal have been mostly studied as a „black box" (ie the main assessed parameter is pollutant removal efficiency), and there is very limited information available about of the pollutant removal mechanisms and process dynamics in these systems.

The aim of this paper is to briefly review basic processes of phytoremediation with special emphasis on rhizoremediation and plant-microbe interactions in plant–assisted biotransformation of organic and inorganic pollutants in soil. In addition, the potential and challenges of phytoremediation strategy for enhanced removal of organic and inorganic pollutants from water in treatment wetlands are addressed.

PHYTOREMEDIATION PROCESSES

According to Cunningham and Berti phytoremediation is defined as the use of green plants to remove, contain, or render harmless environmental contaminants [5]. In this process specially selected or genetically engineered plants are used which are capable of direct uptake of pollutants from the environment [6]. Phytoremediation can be applied to both inorganic and organic pollutants present in solid and liquid substrate [7]. Generally, phytoremediation of contaminants by a plant involves the following steps: uptake, translocation, transformation, compartmentalization, and sometimes mineralization [8]. Factors affecting the uptake, distribution and transformation of organic compounds by a plant are mainly related to the physical and chemical properties of the compound (e.g. water solubility, molecular weight, octanol-water partition coefficient), as well as environmental conditions (e.g. temperature, pH, organic matter, and soil moisture content) and plant characteristics (e.g. root system, enzymes) [9, 10]. Although the designations of different phytoremediation strategies vary in literature, the principal scheme is given in Fig. (**1**).

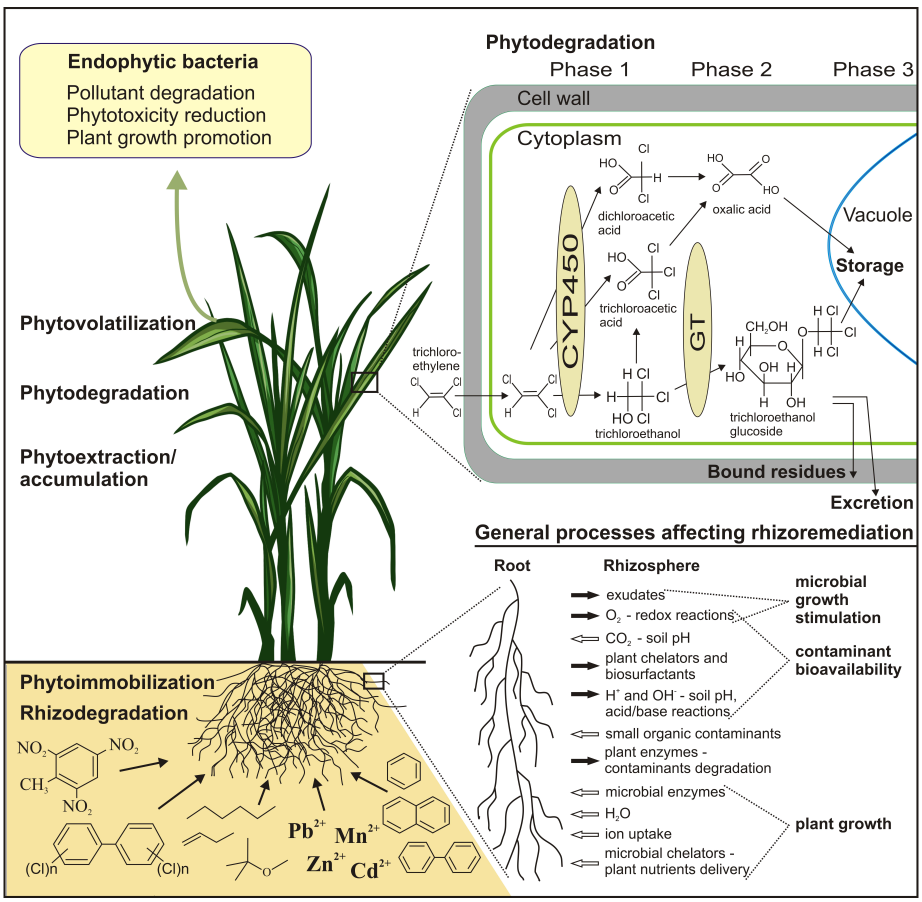

Fig. (1). Phytoremediation of various organic and inorganic pollutants in soil. Plants are capable of removing organic and inorganic contaminants from soil by roots (phytoimmobilization), but also transporting and concentrating them in the harvestable part of the plant (phytoextraction/accumulation). In some cases transpiration to the atmosphere through leaf stomata may follow (phytovolatilization). Organic contaminants can be metabolized inside the plant (phytodegradation) in three sequential steps (phase 1 – transformation, phase 2 – conjugation, phase 3 – compartmentalization) using enzymes, such as CYP450 – cytochrome P450; GT – glycosyltransferase, resulting in the storage of the contaminant in the vacuole, integration into the cell wall, or excretion from the cell. In addition, organic contaminants can be degraded by plant-associated microorganisms in the rhizosphere (rhizodegradation). Plants facilitate the biodegradation of contaminants by releasing root exudates and other compounds to the surrounding soil as well as providing surface for the colonization of microbes, contributing in this way to the increased number and metabolic activity of microorganisms (rhizosphere effect) and enhanced bioavailability of the contaminant. Plants provide nutrients for endophytic bacteria and induce catabolic gene expression. Endophytic bacteria degrade organic pollutants reducing in this way phytotoxicity, and produce plant growth promoting hormones.

Inorganic contaminants (heavy metals and radionuclides) can be either taken up from the soil and immobilized by the roots (phytoimmobilization), or transported to the plant shoot (phytoextraction) [11]. Since under most circumstances the bioavailability of metals (including some metals essential to life) in soil is rather low, plants possess highly effective metal uptake systems using transporter molecules such as zinc-regulated transporter protein, copper transporter protein etc. [12]. In addition, plants are capable of secreting metal-chelating molecules like siderophores and organic acids (malate, citrate), and biosurfactants such as rhamnolipids to the surrounding soil, and also extruding protons from the roots to acidify the soil and mobilize soil bound metals (Fig. **1**) [13, 14]. Contrary to organic pollutants heavy metals cannot be biodegraded inside the plant but are only transformed from one oxidation state or organic complex to another [14]. As a result, metals tend to accumulate in the plant. Nearly 450 hyperaccumulator plants ranging from annual herbs to perennial shrubs and trees (e.g. tobacco, sunflower, mustard, maize, pennycress, brake fern, Russian thistle, rattlebush, python tree, willow, poplar) have been described to accumulate and detoxify extraordinary high levels of metal ions, such as Ni, Co, Pb, Zn, Mn, Cd, etc. in their above ground tissues [15-18]. It has been suggested, that the prevention of herbivory and disease may be the main function of hyperaccumulation for the plant [17, 19]. Still, in this case it is possible to harvest and remove plants from the site after remediation for disposal or recovery of the contaminants [10]. For some inorganic elements (Hg, As, Se) uptake by roots followed by transport to the shoot and transpiration to the atmosphere through the leaf stomata (phytovolatilization) have been observed [16, 20]. Since the volatile forms of Hg and Se are also toxic, it is questionable whether the volatilization of these elements into the atmosphere is desirable or safe [16, 21].

Organic pollutants in soil like chlorinated solvents and polyaromatic hydrocarbons (PAHs) can be taken up and immobilized by plant roots [22] as well as transpired from the shoot (methyl tert-butyl ether – MTBE, trichloroethylene – TCE, ethyl-benzene, xylene) [23, 24]. In addition, contrary to inorganic pollutants plants are capable of metabolizing organic contaminants (phytodegradation). The metabolism of contaminants by a plant can be divided into three phases: transformation, conjugation and compartmentalization (Fig. **1**). In the transformation phase, contaminant is chemically modified (oxidation, reduction, and hydrolysis) and transformed into more polar, water soluble form by enzymes such as cytochrome P450 or carboxylesterases. By conjugation with endogenous molecules like sugars or peptides, the transformed contaminant is made less phytotoxic by glycosyltransferases and glutathione S-transferases, followed by compartmentalization phase where contaminant is transferred to the various compartments of the cell (storage in the vacuole or integration into cell wall) or in some cases excreted from the cell [11, 23, 25]. However, there is a principle difference between metabolism of contaminants by a plant and by microorganisms – most contaminants are not utilized as a source of C, N and energy by plant since plants do not possess complete catabolic pathways for degradation and mineralization of pollutants [25, 26]. During the degradation process even more toxic by-products (from the human point of view) may be produced compared to the initial pollutant. For instance, the transformation of TCE into trichloroethane, or the release of some metabolites from volatile pollutants into the environment by evapotranspiration have been detected [23, 27]. Only a few types of contaminants, for example polychlorinated biphenyls (PCBs), PAHs, nitroaromatics and linear halogenated hydrocarbons can be completely mineralized by plants such as poplar, willow, alfalfa and different grass varieties [15, 28].

Transgenic plants can be developed by transferring genes from organisms which have the potential for degradation/mineralization of xenobiotic pollutants to candidate plants to improve the ability of plants to degrade/metabolize xenobiotic pollutants. Genes involved in the degradation of xenobiotic pollutants can be isolated from bacteria/fungi/animals/plants and introduced into candidate plants using *Agrobacterium* mediated or direct DNA methods of gene transfer [25]. Specific catabolic genes essential for the degradation of a contaminant are overexpressed in a plant, resulting in enhanced phytoremediation. For example, transgenic tobacco, rockcress, mustard, poplar, rice, potato have been reported to be able to improve phytoextraction, phytovolatilization and phytodegradation of heavy metals and organic contaminants like explosives, chlorinated solvents, PAHs, polychlorinated biphenyls, various herbicides, and atrazine [25, 29-31]. The most recent and very promising approach to improve phytoremediation ability is the construction of plants with enhanced secretion of enzymes capable of degrading xenobiotics into the rhizosphere [32, 33]. The advantage of this method is that the plants do not need to take up the pollutants in order to detoxify them; instead, the secreted enzymes can degrade the pollutants in the rhizospheric zone [34]. However, there are strict regulatory restrictions for *in situ* applications of genetically modified organisms in the European Union and promising results have been obtained only in the laboratory and greenhouse experiments.

RHIZOREMEDIATION AND MICROBE-PLANT INTERACTIONS IN PHYTOREMEDIATION

Rhizoremediation (also rhizodegradation, microbe-assisted phytoremediation, rhizosphere bioremediation) utilizes the complex interactions involving roots, root exudates, rhizosphere soil and microbes that result in degradation of contaminants to non-toxic/less-toxic compounds. Plant roots stimulate rhizosphere microbial communities by aerating the soil and releasing exoenzymes as well as nutrients through root exudates while also providing surface for colonization and niches to protect bacteria against desiccation and other abiotic and biotic stresses [28]. Rhizospheric microorganisms in turn promote the plant growth by nitrogen fixation, nutrient (i.e. phosphorus) mobilization, production of plant growth regulators, decreasing plant stress hormone levels, providing protection against plant pathogens and degradation of pollutants before they negatively impact the plant (Fig. **1**) [35, 36]. Consequently these mutual interactions, also known as rhizosphere effect, result in elevated number, diversity and metabolic activity of microbes able to degrade contaminants or support plant growth in close vicinity of roots compared to bulk soil [37, 38]. In many cases, rhizosphere microbes are the main contributors to the contaminant degradation process.

The amount and composition of root exudates which create nutrient rich environment in the vicinity of roots is specific to plant family or species. Root exudates contain organic acids (lactate, acetate, oxalate, succinate, fumarate, malate, and citrate), sugars and amino acids as main components but also secondary metabolites (isoprenoids, alkaloids, and flavonoids) which are released to the soil as rhizodeposits [39- 41]. The main fraction of exuded organic acids are present in soil as dissociated anions (carboxylates) [42]. It has been suggested that 10-44% of the photosynthetically fixed carbon is excreted by rhizodeposition [43, 44]. Root exudates can be used as an energy source by microorganisms. In addition, the structure of many secondary metabolites resembles those of contaminants thus inducing the expression of specific catabolic genes in microorganisms necessary for the degradation of the contaminant [40]. For instance, plant secondary metabolite salicylate has been linked to the microbial degradation of PAHs (naphthalene, fluoranthene, pyrene, chrysene) and PCB [45-47], while terpenes can induce the microbial degradation of toluene, phenol, and TCE [48]. Easily degradable root-exuded compounds can also serve as co-metabolites in processes where contaminants cannot be used as a sole carbon source (i.e. aerobic degradation of trichloroethylene [49] due to negative energy balance) [11]. This is important under many circumstances where microorganisms cannot rely on energy gain from the contaminant and cometabolism is the only route for the degradation of contaminant. Plant roots along with some rhizospheric bacteria may also excrete biosurfactants thus increasing the bioavailability and uptake of pollutants [28, 50, 51]. This aspect can be especially beneficial in aged soils with low contaminant bioavailability that generally appear to be much less responsive to rhizodegradation than freshly spiked soil [52, 53].

A recent strategy to improve phytoremediation and detoxification of contaminants is the use of endophytic bacteria. Endophytic bacteria are described as non-pathogenic bacteria and they seem to have a ubiquitous existence in most if not all higher plant species. They often belong to genera commonly found in soil, including *Pseudomonas*, *Burkholderia*, *Bacillus* and *Azospirillum* [54-56]. Endophytic bacteria are also known to have plant growth promoting and pathogen control capabilities [57, 58]. A major advantage of using endophytic bacteria over rhizospheric bacteria in phytoremediation is that while a rhizospheric bacterial population is difficult to control, and competition between rhizospheric bacterial strains often reduces the number of the desired strains (unless metabolism of the pollutant is selective), the use of endophytes that naturally inhabit the internal tissues of plants reduces the problem of competition between bacterial strains [59, 60]. Studies suggest that these bacteria can be used to complement the metabolic potential of their host plant through direct degradation [61-64] as well as transfer of degradative plasmids to other endophytes [65, 66]. In addition to pollutant degradation pathway endophytic bacteria may also possess the capability to enhance plant growth and adaption by 1-aminocyclopropane-1-carboxylate (ACC)-deaminase activity, siderophore production and nutrient solubilization. Presence of such bacteria in plants leads to more efficient phytoremediation activity, and reduces need for additional fertilization [67].

Even enhanced rhizoremediation might be considerably slower than *ex situ* treatments due to environmental restrictors at field sites such as competition by weed species which are better adapted to the site [68], limited plant growth in heavily and unevenly contaminated soil, presence of plant pathogens and other biotic and abiotic stressors [33]. Furthermore, rhizoremediation is effective only in rooting zone and is unsuitable for usage in deeper subsurface layers. Some toxic contaminant metabolites can also bioaccumulate in plants making strict regulations of plant material treatment necessary. However, despite the aforementioned shortcomings rhizoremediation is emerging as one of the most effective means by which plants can affect the remediation of organic contaminants, particularly large recalcitrant compounds [11, 33, 42, 69]. Besides its relatively low maintenance costs, no size restrictions for the area and environmentally friendly nature, the quality and texture of soil is also improved by additions of organic materials, nutrients and oxygen *via* plant and microbial metabolic processes. Despite the challenges of introducing phytoremediation from lab and greenhouse scale to field, rhizoremediation has been used to treat field sites contaminated with petroleum hydrocarbons [68, 70], PAHs [71, 72], TNT [73], BTEX [74] and TCE [75, 76]. To date, many successful cases of phytoremediation of various organic contaminants using rhizospheric or endophytic bacteria have been reported (Supplementary Table **1**).

Likewise to other bioremediation techniques detailed and continuous monitoring of chemical and biological indicators is essential to ensure phytoremediation process efficiency and environmental safety.

APPLICATION OF PHYTOREMEDIATION IN TREATMENT WETLANDS

Treatment wetlands (also known as constructed wetlands) are effective and low-cost operational alternatives to conventional technologies for the elimination of a wide range of contaminants from wastewaters and polluted groundwater [77-80]. Generally treatment wetlands have been applied to treat municipal or industrial wastewater [81], and more recently for the removal of excessive nitrogen and phosphorous from polluted surface and subsurface waters to protect aquatic ecosystems [82, 83]. An integration of phytoremediation has been suggested to improve the performance of existing wastewater treatment in constructed wetlands, especially towards the emerging micropollutants, i.e. organic chemicals, personal care products and pharmaceuticals (incl. antibiotics) [84, 85]. However, the biogeochemical processes associated with the transformation of the organic chemicals in vegetated treatment wetlands are so far rarely evaluated probably owing to the complex and synergistic nature of ongoing processes. In a complex treatment wetland system several elimination pathways of organic compounds (volatilization, photochemical oxidation, sedimentation, sorption, and biodegradation) may occur simultaneously while plants may contribute either by direct contaminant uptake and accumulation, phytovolatilization, and metabolic transformation, or by creating conditions favourable for pollutant removal within the treatment systems [86]. The latter involves acting as suitable surface for biofilm anchorage, promoting the development and growth of different microbial species within the systems by secreting root exudates, pumping and releasing oxygen to the deeper layers of the wetland media, retaining suspended solids particles and insulating against low temperature [87]. The relative importance of a particular process varies, depending on the organic or inorganic contaminant to be treated, the treatment wetland type (free-water, subsurface flow, horizontal flow or vertical flow, type of vegetation) and operational design (wastewater loading rate and retention time, soil matrix type). Zhang and co-workers analysed how much different processes such as microbial degradation, photodegradation and plant uptake contribute to the removal of pharmaceutical compounds from wastewater in aquatic plant-based systems [88]. They found that plant uptake played the dominant role in elimination of clofibric acid and caffeine, and was also significant in the case of ibuprofen. However, the impact of the plants presence and the ability of particular species to improve the removal efficiency of certain pharmaceutical compounds and personal care products still remains unclear. This is because many other factors, like the structure of rhizosphere microbial communities and the properties of the wastewater, as well as environmental (temperature, availability of electron acceptors) and operational conditions (hydraulic retention time, specific surface area, loading mode) may all act in concert [89, 90]. For example in case of surface flow constructed wetland planted and unplanted mesocosms were not significantly different in their abilities to remove pharmaceuticals [91].

Besides their role in wastewater treatment, macrophytes also have the ability to mitigate pesticide pollution arising from various agricultural non-point sources in treatment wetlands [92, 93]. For instance, wetland treatment of water contaminated with low chlorinated benzenes has been investigated in several studies [94, 95] and it has been shown that although the uptake of this compound by plants was <0.1% of the initial concentration, the mean removal of hexachlorobenzene (HCB) was higher in the vegetated microcosm wetlands [96]. Furthermore, the dechlorination rates of HCB were found to be higher in sediment layers with well-developed root zones [97]. In case of herbicide S-metolachlor the removal efficiency have been found to be dependent on hydraulic regime in planted subsurface flow constructed wetland being substantially lower in continuous flow operation mode compared to batch mode [98]. The batch operation strategy also improved the removal efficiency of ibuprofen, diclofenac, oxybenzone, caffeine, salicylic acid, ketoprofen and bisphenol A in planted horizontal flow constructed wetland systems [99, 100].

Vegetated treatment wetlands also offer a promising way to remediate water contaminated with inorganic compounds like metals and metalloids with metal uptake by various macrophytes being the prominent pollutant removal mechanism. For instance, common reed *(Phragmites australis)* has a potential to extract and accumulate chromium from tannery wastewater [101, 102] while broad-leaved cattail *(Typha latifolia)* has been established as a prominent copper and cadmium remover from industrial wastewater in a lab-scale experiment [103]. In another lab-scale study heavy metal (Cd (II), Hg (II), Cr (VI) and Pb (II)) removal from a synthetic landfill leachate ranged from 92 to 98% in all reactors planted with wild cane *(Gynerium sagittatum)*, taro *(Colocasia esculenta)* and Parrot's flower *(Heliconia psittacorum)* [104]. Good pollutant removal efficiencies achieved in lab-scale do not always guarantee treatment process success in larger/field scale; still, a few successful phytoremediation treatment trials of inorganic contaminants in constructed wetlands have been reported. A pilot–scale study in a subsurface flow treatment wetland showed a great potential of the aquatic macrophyte southern cattail (*Typha domingensis)* for the phytoremediation of water contaminated with mercury. Also, in a study by Anning and co-workers the removal efficiencies for different heavy metals from a contaminated river water varied (∼20–77%) in a planted treatment wetland system [105]. The removal of heavy metals in treatment wetlands could be enhanced by supplementing treatment systems with siderophore-producing bacteria as shown in a study where repeated bioaugmentation increased the amount of Cu extracted by common reed (*Phragmites australis)* twice due to the increased Cu bioaccessibility in the rhizosphere [106].

CONCLUSIONS AND OUTLOOK

Phytoremediation is a technology that is based on the combined action of plants and their associated microbial communities to degrade, remove, transform, or immobilize toxic compounds located in soils, sediments, ground water and surface water. Phytoremediation has been used to treat many classes of contaminants including petroleum hydrocarbons, chlorinated solvents, pesticides, explosives, heavy metals and radionuclides in soil and polluted water. There are several advantages of phytoremediation compared to conventional techniques, such as low cost, low disruptiveness to the environment, public acceptance, and potentiality to remediate various pollutants. In addition, plants as autotrophic systems with large biomass require only a modest nutrient input, and they also prevent the spread of contaminants through water and wind erosion [29]. Candidate plant for phytoremediation should have the characteristics such as high biomass production, extensive root system, and ability to tolerate high concentration of pollutants and withstand environmental stress. Like other treatment technologies, phytoremediation has its disadvantages e.g. climatic and geological limitations, potential phytotoxicity of the contaminant, potential for the contaminant or its metabolites to enter the food chain, and potentially longer timescale compared to other technologies [6]. Although some success has been reported using plants alone in bioremediation, the use of plants in conjunction with plant associated (rhizosphere or endophytic) bacteria offers more potential for bioremediation. The implementation of treatment wetlands for phytoremediation of wastewater or polluted water originating from various sources allows removing organic and inorganic pollutants from water in an environmentally friendly and economically feasible way. Treatment wetlands utilizing phytoremediation approach could be combined with existing treatment systems, or the complex treatment wetland systems could be designed according to the site-specific requirements. However, compared to phytoremediation of polluted soils the mode of action and technological aspects of the plant application for the pollutants removal in treatment wetlands is less studied, especially in case of inorganic compounds and micropollutants (pharmaceutical compounds and personal care products). Also, the possible release of pollutant transformation products and unknown derivatives from treatment wetlands into environment has to be taken into account. Further research is needed in order to understand the plant-microbe interactions during removal of contaminants including micropollutants in different types of treatment wetlands. Based on this knowledge treatment wetland design and operational parameters could be improved to achieve more efficient pollutant removal efficiency.

CONFLICT OF INTEREST

The authors confirm that no conflicts of interest are associated with this article.

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SUPPORTIVE/SUPPLEMENTARY MATERIAL

Supplementary Table **1**. Examples of successful phytoremediation cases of various organic contaminants using rhizospheric bacteria (RH) or endophytic bacteria (EN).

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