

Constructed Wetlands and their Role in Remediation of Industrial Effluents *via* Plant-Microbe Interaction – A Mini Review

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Abstract

Phytoremediation has proven to be an efficient, widely used and adopted method for the treatment of wastewaters and soil in the form of Constructed Wetlands (CWs). Plant-microbe interaction can be utilized to overcome the increasing health hazards due to the increase in pollution. Plants have the ability to live in a mutual relationship with many different kinds of microbes. Most of them live and interact with the roots of plants and that part of the plant seems very important in overcoming the conditions of less water, excessive evapotranspiration, rapid nutrient loss etc. Microbes, mostly bacteria interact with the roots of the plants and degrade the minerals and materials present in the soil in order to obtain energy for themselves. Plants get benefit from the microbial activities as they can utilize the minerals degraded by the root-colonizing bacteria. Root-colonizing bacteria can either be endophytic or ectophytic. The main focus of this review is on the bacteria that synergistically help the plant to degrade the minerals present in the surrounding pollutants and in return gets shelter and exudates from the plants. Removal of industrial effluents from the soil and water is necessary because they seem to have been causing several toxicity problems in the soil microbiota, plants as well as in the humans that consume them.

Keywords: Constructed wetlands; Industrial effluents; Waste-water; Synergism; Plant-microbe interaction

Introduction

Constructed wetlands (CWs) are an alternative method for wastewater treatment and its purification. In the past decades, CW techniques were hardly used for contamination removal [1,2], although first time in early 1950s, Dr. Kathe Seidel used constructed wetland for wastewater treatment [3]. But evolutionary period started since the 1990s, this technique is used to treat various types of wastewaters i.e., industrial effluents, municipal wastewater, and drinking waters. CWs are the engineered systems that mimics the natural processes by removing the pollutants or by reducing the level of pollutants to a dischargeable limit [4].

Constructed wetland treatment systems efficiently remove several kinds of pollutants from wastewater, drinking water, and industrial effluents [5-7] within the controlled environment and are considered as environment-friendly. Removal of effluents, metal and waste are performed through a variety of processes i.e., by increasing sorption, hydrolysis, filtration and oxidation, precipitation, binding with iron oxide, microbial activity and uptake by plants. In response to removing pollution, constructed wetlands differ in their processes, cost effectiveness and their design [8] and also enhance the waste removal performance by using different substrates i.e., Phosphorus (P) [9]. In the recent years, scientists have tried to remediate or clean up wastewater through phytoremediation and bioremediation. Constructed wetland treatment systems use microbes and rooted plants to remove contaminants from soil or wastewater. It takes

advantage of natural wetland processes (biological [10], physical and chemical processes [11]) to remove contaminants [12] but the efficiency of all the processes (Chemical, physical and Biological) differ with water residence time.

This technology is now widely used (in America, China, Argentina, Czech Republic, Greece, Netherlands, and Europe [13-15]) and tested to efficiently improve water quality. However, the industrial and environmental sector pay more attention to remove heavy metals from industrial effluents using CWs [7,16,17]. CWs are more effective and have significantly low capital costs as compared to conventional system (ITRC 2003) and also require less labor and electricity to operated (USEPA 1988). The main objective of constructing the constructed wetland is to optimize the interaction of the substrate with microbial species and plants cells and then bioconversion into harmless products.

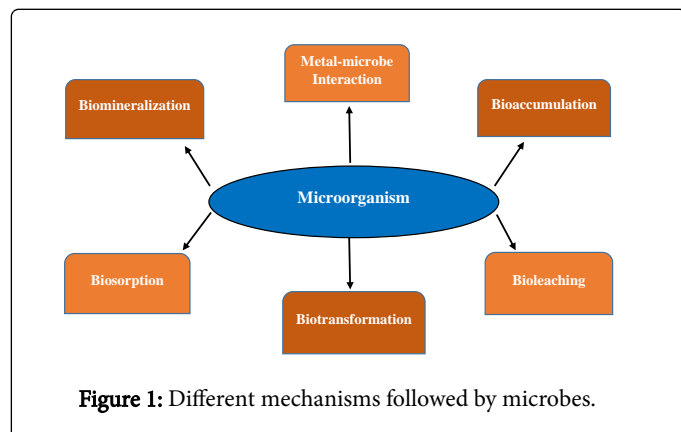
The haplotype plants and microbes that are used in wastewater treatment not only accumulate heavy metals but also perform the function of catalysts for purification reaction [18]. Several factors also influence the remediation process which includes plant transpiration, and growth rates, storage, and accumulation in root and leaf cells, sedimentation, pH of media [7,19]. CWs are composed of one or two chambers which are filled with the substrate that support the growth of haplotype plants and microorganisms. The substrate is both directly and indirectly involved in the remediation of pollutants, directly these substrates are involved in precipitation and filtration of suspended solids, sorption of heavy metals and organic matter [20] and indirectly act as adhesion of microorganisms and support to root system [21]. Nowadays an integrated technique is used to efficiently remediate the contaminants or pollutants from drinking water and industrial effluents from the soil [11,22,23]. The main aim of this review is to

evaluate and explain the role of constructed wetlands to remove industrial effluents [19].

Plant-Microbe Interactions for Bioremediation – Basis of Constructed Wetlands

Bioremediation is an innovation, and a promising technique to degrade and recover heavy metals that are present in contaminated lands and water [24]. It is the way to employ life forms or to eliminate contamination. It is important to comprehend that this remediation requires no chemicals at all, until unless it uses a living being that causes adverse effects in certain situations [25]. Different forms of bioremediation in practice are the microbiological approach with one or many contaminant specific degrading microorganism which includes augmentation of contaminated sites. Also, the approach of microbial ecology is in use which includes certain chemical and physical parameters at a site to refine the degradation [26]. The sites that are loaded with natural waste materials, microbes including parasites and different protists, continue to metabolize organic matter to degrade waste material [27].

Since microbes developed different strategies (shown in Figure 1) for surviving in contaminated habitats as these microorganisms are known to adopt various detoxifying mechanisms like bioaccumulation, biosorption, biomineralization, and biotransformation, as they can be capitalized for bioremediation either by ex-situ or in-situ strategies [28].



Microbes are omnipresent dominating in heavy metal contaminated lands and produce non-toxic forms of heavy metals [24,29]. Microorganisms remove contamination (heavy metals) from the soil by growth promoting substances like chemicals while different methods for restoration of the environment are oxidation, immobilization, volatilization and last but not the least, the transformation of heavy metals [30]. By understanding growth mechanism of microbes through designer microbe approach, by the activity of microbes in the contaminated site and by the microbial response to the environment, bioremediation can be made successful [30]. For example, energy dependent and plasmid-encoded metal efflux systems which involve ATPases and also the chemiosmotic proton pumps, are described for Cr, Cd and as resistance in many bacteria [31]. This new emerging science in which plants being the sole source to remediate the contaminated water and soil, have paved the new avenues for research in this particular field [32]. Plants and microbes are the sunlight-driven hotspots for turnover of organic compounds. Various microbes present in the rhizosphere are of great

significance in the sustainability of soil and also in nutrient cycling [33]. Plant-microbe mediated bioremediation (Figure 2) is the feasible technique to clean contamination as several plants can get beneficial effects in result from synergistic interaction with plant-growth promoting Rhizobacteria (PGPR). Such interaction enhances plant growth, alleviate toxicity and increase heavy metal tolerance [34]. Synergistic association benefits both, plants and the microbes, plants through nutrient uptake and reduced toxicity of contaminants, and microbes through the allocation of nutrients via root exudates [35]. Remediation using plants is not yet launched on commercial level due to its major drawback of slow processivity and limited availability of contaminants as it is affected by climate but by focusing on plant-microbe interaction, such drawbacks can be avoided. Environments contaminated with recalcitrant organic compounds can further be remediated by the interaction of plant and microbes in the rhizosphere, this all in-turn extends the scope of remediation technologies [35]. Aside from mutualism, the interaction of plant-microbe influences the quality of water at Rhizospheric level providing capacity for mitigation of contamination [36].

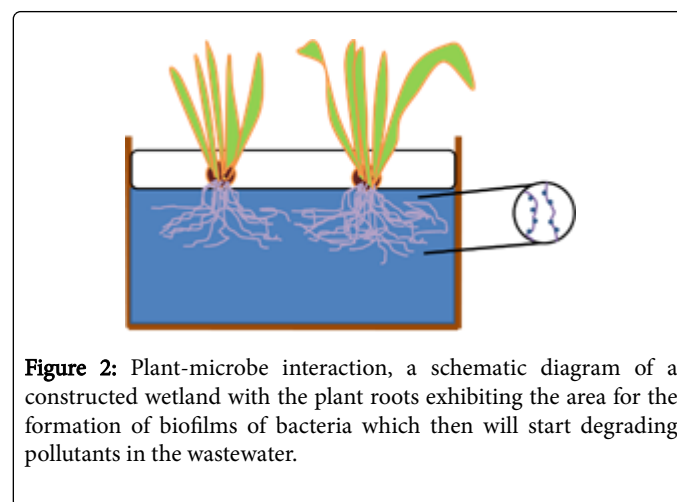


Figure 2: Plant-microbe interaction, a schematic diagram of a constructed wetland with the plant roots exhibiting the area for the formation of biofilms of bacteria which then will start degrading pollutants in the wastewater.

Industrial Effluents and types of Industrial Effluents

Industries are established for the well-being of humans and to fulfill the needs of a continuously growing population. Industries, on one hand, produce useful products but at the same time, they are a major source of environmental contaminants. Industries are polluting the environment through their wastewater discharge, atmospheric deposition and the improper management of effluents, generated due to anthropogenic activities, creating the most discerning problem of developing countries [37,38]. The problem has become more exacting because of the unsafe disposal of effluents into the environment [39]. Contamination of water bodies by industrial effluents is an emerging challenge for crowded countries [40]. Effluents discharged into water bodies and on land are serious hazards for humans and for the sustainability of ecosystem [37]. Water pollution was confined to small areas till the mid of 18th century. With the advent of industrial revolution, the development of different petroleum fuelled industries and the internal combustion engine, a large amount of fresh water is used for the production and cooling purposes. This leads to the addition of various kinds of raw materials, waste products, and intermediates into the water which in turn causes water pollution [41].

The hallmark of wastewater is its color, odor and turbidity as well as its organic and inorganic contaminants. Organic contaminants could

be dissolved or undissolved volatile organic compounds (VOCs) including phenols, chlorobenzene, and hydrocarbons. While sulfides, trace minerals, chlorides, nitrogen and phosphorous are inorganic pollutants found in wastewater [42]. The potential source of organic pollutants is soaps, detergents, and associated chemicals. Hydrogen Sulfide, H₂S, is an inorganic pollutant, resultant of anaerobic decomposition of organic matter consisting of sulfur. Wastewater coming from paper mills, chemical plants, textile mills, and tanneries is the source of H₂S [43].

In Pakistan, textile, cement, metal, chemical dye, fertilizer, pesticide, tanneries and petrochemical industries etc. are adding to water pollution. These pollutants cause an increase in biological oxygen demand (BOD), chemical oxygen demand (COD) of water bodies. Industrial effluents also add heavy metals like cadmium, chromium, nickel and lead to the fresh water sources making them unfit for aquatic life, irrigation and drinking purposes. It has been reported that about half of the population of developed countries has no access to clean drinking water [44]. Pollution strength and flow of industrial wastewater or effluents vary greatly [45].

A large quantity of water is consumed by textile industry and a massive quantity of chemicals is used in different processes like scouring, bleaching, dyeing etc. Thusly a large amount of fresh water is converted to colored water; full of effluents [46]. This wastewater containing dyes is a major threat to the environment due to high toxicity level. The dyes are classified into six different classes i.e., Azo, sulfur, indigoid, anthraquinone, phthalocyanine and triphenylmethane. The aromatic nature of these dyes make them mutagenic and carcinogenic [47,48] and hence threatening the survival of humans and aquatic biota [49]. The worldwide annual production of dyes is 1 million tonnes and more than 10% of it is discharged by textile industry [50,51].

Ammonia released from agriculture-based industries is one of the major causes of eutrophication and acidification of water bodies [52]. Effluents from phosphorus and fertilizer industries are globally problematic. These effluents are enriched with acids and heavy metals, which are a substantial source of environment and underground water contamination [53]. Effluents from agro-food industry are a continuous source of water pollution [54].

Various kinds of heavy metals are significantly found in industrial wastewater. Heavy, metals are present in source as well as in treated water, posing a serious threat to public health [55,56]. The metals having atomic number greater than 63.5 and less than 200.6 are called as heavy metals [57]. Industries like paper industry, metal plating facilities, fertilizer industry and mining operations etc. discharge heavy metals into the environment. Heavy metals like zinc, copper, mercury, lead, chromium and cadmium are of particular interest. Zinc and copper are essential for human health in trace amounts but their higher concentration can cause serious health problems and even death [58,59]. Nickel can lead to lungs and kidney disease if exceeding a critical level and it is a known human carcinogen [60]. Lead is a neurotoxin as well as damages liver, reproductive system and kidney [61]. Chromium exists in two multivalent forms i.e., Cr (III) and Cr (VI). Cr (VI) is more toxic than Cr (III), because it is more water soluble, permeable to biological membranes, has the ability to accumulate in the food chain and is carcinogenic [62,63].

Removal of Pesticides and Lignin Using Constructed Wetlands

Several organic pollutants are constituent of industrial wastewaters, i.e., pharmaceuticals and personal care products (PPCP), pesticides, veterinary drugs etc. [64]. Chemical plant protectors are a serious threat to environment i.e., the threat to the life of several organisms and cause of pollution [65]. Conventional treatment methods are inefficient in removing such organic pollutants [66]. Whereas constructed wetlands are quite efficient in removing organic pollutants [67]. Typha and Phragmites are considered good for decontamination of wastewaters from organic pollutants through CWs. Conventional parameters for PPCP and pesticides are mentioned in the Table 1 given below [71].

Serial No:	Parameters	Methods for determination	References
1	NH ₃	Dehydrogenase assay	[68]
2	TSS	Turbidimeters	[69]
3	BOD	BOD biosensor	[70]
4	pH	Selective Electrode	[71]
5	Temperature	Digital Thermometer	[71]
6	Dissolved Oxygen	Digital Oxymeter	[71]

Table 1: Parameters for Pharmaceuticals and Personal Care Products.

Extensive research has led to the development of bioremediation techniques and their application [72]. Plant-endophytic alliance might be an appropriate method to restore contaminated ecosystems. This relation can be improved by introducing novel catabolic/metabolic genes into any of the partners. The potential obtained by engineered organisms to degrade or accumulate contaminants is quite high than their wild relatives [73]. Constructed wetlands, another way for removal of pesticides from water sources [74]. Surface flow constructed wetland (SFCW) are considered best for removal of pesticides [75,76]. Introduction of bacteria can be used to enhance the efficiency of constructed wetlands for remediation of the industrial effluents [77]. Constructed wetlands may use the influence of hydraulic residence time and vegetation density to sediment pesticides e.g., chlorpyrifos. Wetlands, however cannot remove diazinon effectively, as has low sorption to sediment particles [78].

Phenolic compounds are also present in wastewaters in form of phenol, bisphenol, 4-tert-butylphenol etc. These can be removed by using Phragmites australis in CW coupled with BPA-degrading and 4-t-BP-degrading bacteria. Major removal procedure might involve adsorption by initial removal followed by biodegradation [79].

The sixth largest source of industrial pollution is paper and pulp industry. Approximately 20-250 m³/t is the rate of air dried pulp in wastewaters [80]. Efficiency of CWs for removal of BOD COD and chlorophenols from pulp has been reported [81]. According to a study, COD and color reduction from pulp is efficient via horizontal sub-surface constructed wetlands with use of *Canna indica* [82].

Removal of Heavy Metals (Zn, Pb, Cu, Silver, Nickel, and Cadmium) Using Constructed Wetlands

Contamination of soil and groundwater by heavy metals may cause serious and long-lasting health risk to humans and environment due to their toxicity. Because the addition of heavy metals in soil badly affects its physicochemical properties which may cause infertility and low yield of crops, moreover heavy metals present in the soil can enter into the food chain and continuous leaching of heavy metals can contaminate the ground water. The monitoring of heavy metal pollution in soil and related groundwater is of high significance in the areas near to the industries [83]. Due to the release of great quantity of metal-polluted wastewater, industries bearing heavy metals, such as Cd, Cr, Cu, Ni, As, Pb, and Zn, are mainly dangerous and hazardous among the chemical-intensive industries. These heavy metals are high soluble in the aquatic environments, so living organisms can absorb these heavy metals. If these heavy metals get entry in the food chain, large concentrations of heavy metals may accumulate in the human body. If the metals are ingested beyond the permitted concentration, they can cause serious health disorders. Therefore, it is compulsory to treat metal contaminated soil and wastewater before its discharge to the environment [84].

Any toxic metal may be called heavy metal, irrespective of their atomic mass or density. Heavy metals are affiliated with a nonspecific division of elements that show signs of metallic properties. These consist of transition metals, some metalloids, lanthanides, and actinides. Any metal species may be considered a “contaminant” if it occurs where it is unwanted, or in a form or concentration that causes an unfavorable human or environmental consequence. Metals/metalloids comprise of lead (Pb), cadmium (Cd), mercury (Hg), arsenic (As), chromium (Cr), copper (Cu), selenium (Se), nickel (Ni), silver (Ag), and zinc (Zn). Other less common metallic contaminants include aluminum (Al), cesium (Cs), cobalt (Co), manganese (Mn), molybdenum (Mo), strontium (Sr), and uranium (U).

Heavy metals are innate constituents of the earth's crust, but haphazard human actions have severely distorted their geochemical cycles and biochemical balance. This results in an increase of metals in plant parts having secondary metabolites, which may cause particular pharmacological activities. Long-term exposure to heavy metals such as cadmium, copper, lead, nickel, and zinc can cause harmful health effects in humans [85].

The sufficient protection and restoration of soil ecosystems contaminated by heavy metals need their characterization and remediation. Remediation is normally focused on the collection of regulatory requirements [86]. To get the concentration of heavy metal content in the soil, acid digestion of soil can be used because mineral matrix binds the heavy metals strongly. Acid digestion of soil is new practical approach and gives a true depiction of total quantity of selected heavy metals in the soil. Soil was believed the most polluted compartment of the environment if it has 3% of total metal concentration [83].

Bioremediation technique is presenting an impressive exchange of predictable expensive and inefficient methods for the heavy metal removal. Bioremediation is the ‘green’ essential method for the heavy metal elimination without creating secondary metabolites in the ecosystem. The metabolic prospective of several bacterial, algal, fungal as well as plant species has the ability to eradicate the heavy metals from the infected sites. Variety of strategies like bioaccumulation, biosorption, biotransformation, rhizofiltration, bioextraction and

volatilization are in use for removal of heavy metals by the biological species [87]. Biosorption is considered a potential instrument for the removal of metals from waste solutions and for valuable metals recovery, an alternative to the conventional processes, such as those based on ion exchange, or adsorption on activated carbon [88].

Phytoremediation, also called green remediation, botanoremediation, agro remediation, or vegetative remediation, can be known as an *in situ* remediation approach that uses vegetation and associated microbiota, soil amendments, and agronomic practices to eliminate contaminants. The suggestion of using metal-accumulating plants to remove heavy metals and other compounds was first introduced in 1983. Plants may break down or degrade organic pollutants or eradicate and alleviate metal contaminants. Phytoremediation methods for metal contaminants are slightly different from those methods that are used to remediate the sites polluted with organic contaminants. Phytoremediation is energy competent, aesthetically satisfying method of remediating sites with low to-moderate levels of contamination, and it can be used in combination with other more traditional remedial methods as a finishing step to the remedial process. The advantages of phytoremediation compared with classical remediation are that (i) it is more economically feasible using the same tools and supplies as agriculture, (ii) it is environment friendly and does not engage with waiting for new plant communities to recolonize the site, (iii) disposal sites are not required, (iv) it is more likely to be accepted by the public as it is more aesthetically pleasing than traditional methods, (v) it avoids excavation and transport of polluted media thus dropping the risk of spreading the contamination, and (vi) it has the ability to treat sites polluted with more than one type of pollutant. Potentially useful phytoremediation technologies for remediation of heavy metal-contaminated soils include phytoextraction (phytoaccumulation), phytostabilization.

Phytoextraction is the method where plant roots uptake metal contaminants from the soil and translocate them to their above soil tissues. Plants that are used for phytoremediation needs to be heavy-metal tolerant, grow rapidly with a high biomass yield per hectare, have high metal-accumulating ability in the foliar parts, have a plentiful root system, and a high bioaccumulation factor. Two approaches have been proposed for phytoextraction of heavy metals, namely, continuous or natural phytoextraction and chemically enhanced phytoextraction. Continuous phytoextraction is based on the use of natural hyper-accumulator plants with exceptional metal-accumulating capacity. Hyper-accumulators have numerous beneficial characteristics but may have a tendency to be slow growing and generate low biomass, and years or decades are required to clean up contaminated sites. To overcome these shortfalls, chemically enhanced phytoextraction has been developed. This technique involves the use of high biomass crops that are induced to take up large amounts of metals when their mobility in soil is enhanced by chemical treatment with chelating organic acids.

Phytoremediation is best-offered technology for cleaning up heavy metal contaminated soils but have been mostly demonstrated in developed countries. This technology is recommended for field applicability and commercialization in the developing countries also where agriculture, urbanization, and industrialization are leaving a legacy of environmental degradation [86].

Removal of Oil from Water Using Constructed Wetlands

Oil removal from water has been a challenge for the researchers for many years. Now with the advancements in the field of science has made it possible to overcome such problems that have been the issue for mankind for many centuries. Phytoremediation has shown the promise of removing oil contents and hydrocarbons from the soils by the association of the different kinds of the root-colonizing endophytic bacteria [89-91]. Constructed wetlands have been proven to be the source for the removal of different kinds of industrial effluents like, pesticides, heavy metals (Copper, Zinc, Nickel etc.), including many organic and inorganic wastes along with the removal of oils, not only from the soil but also from the waters harboring them. As the constructed wetlands involve the plant-microbe interaction, phytoremediation recently has been used in Pakistan to remove oil contents from the soil contaminated with the industrial discharge of the diesel oils and crude oil [83,92-94]. Constructed wetlands have been used by scientists to remove oil from water in various countries like, China [95], US [96], Malaysia [97] etc. In China, Surface Flow Constructed Wetlands have shown a great promise in removing mineral oil content from the water by promoting the growth of different types of the oxygen-thriving bacteria as a result of the oxygen released in the vicinity of the roots of the plants [95]. Moreover, from the soils, the diesel oil removal has been carried out by the researchers at the National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan. Afzal [92] showed that by using the ryegrass plants, growing in association with the plant growth-promoting bacterial strain *Burkholderia phytofirmans* PsJN, such plant growth-promoting microbes can lead to the efficient growth of the ryegrass plants in the soils contaminated with various kinds of pollutants leading to the ease in the degradation of the pollutants present in the soil [92]. Another kind of constructed wetland system called reverse osmosis constructed wetlands has been used in the US to remove the salt contents from the water and make it available for the irrigation. Similarly, in Malaysia, a new phytoremediation system called floating Vetiver system was used to remove the palm oil mill effluents from the water [97]. Darajeh [97] Used Vetiver grass as the plant source for removal of the palm oil effluents. Vetiver grass has been used widely for phytoremediation for its higher absorption and degradation of the water contaminants [98,99]. Subsurface flow constructed wetlands have also been used in Singapore for the removal of effluents from the waste-waters.

Conclusion

Phytoremediation in the past years has shown a promising evidence in case of the removal of different kinds of industrial effluents from the soils as well as the waters. Removal of contaminants discharged from the industries is essential for the better sustenance of the ecosystem and the protection of the various life forms including, humans, animals, and the microbial life forms. Removal of these contaminants discharged from the industries is of extreme importance and is the most focused area research for the environmental scientists of today because of the increase in the industrialization as well as the population of the developing and under-developed countries has made it an utter requirement for the wealthy people to establish industries and fulfill the increasing demands of the increasing world population. Phytoremediation is the cheap and the most accurate and widely adopted method to remove industrial effluents from the water as well as the contaminated soils. Plant-microbe interaction in most cases

does not work efficiently as it is needed to, in that case, the scientists are making their efforts to engineer certain plant growth-promoting and contaminant degrading bacteria which would harm the environment and the plants to a neglectable level and would efficiently degrade the contaminants present in the soils and in water. Several plant species like *Lolium multiflorum* (Italian ryegrass), *Typha domingensis* (Southern cattail), *Vetiver grass*, *Lolium preenne*, *Brachaiaria mutica* etc. are known as the best for the phytoremediation which work efficiently in association with the microbes colonizing their roots. Proper plant-microbes interaction strategies and the management of the floating wetlands could aid in the development of such wetlands that can be used to treat the greater surface area of water and remove as much contaminant s as possible that could not harm the life forms thriving in the water or in the soil which would otherwise die off due to intolerable level of pollutants in their surrounding environment.

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