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Phylloremediation of Polyaromatic Hydrocarbonic Pollutants Using Phylloshere Bacteria

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Abstract

Polyaromatic air pollution is a serious environmental issue in the modern world due to the high carcinogenicity and geno-toxicity of these pollutants to the all-living beings. These pollutant concentrations in the air are being increased day by day due to huge vehicular emissions, oil refining processes and other industrial processes spread among urbanized areas. Gas or particle bounded PAHs in the air deposit on ground level through wet deposition or dry deposition. These pollutants deposition mainly on plants other than many exposing surfaces spread among the earth. Therefore, the plant leaves highly abundant in urban areas rich with these deposited pollutants. All plant leaves are great niches for microorganisms which is called phyllosphere. Phyllosphere of plants consists with many phyllosphere microorganisms belong to different group of bacteria, fungi, algea and protozoa. Among them bacteria are the predominant phyllosphere microorganisms. The phyllosphere bacteria highly abundant in polluted areas have special capability to degrade polyaromatic hydrocarbons. These PAH degrading phyllophere microorganisms can be used to clean the polluted air, this is called phylloremediation. Phylloremediation is an effective bioremediation method which can use to remediate the air, water and soil polluted from the PAH compounds. The aim of this review is to discuss polyaromatic hydrocarbonic air pollution and deposition of these chemicals on phyllosphere. High depositions of PAHs on plant leaves created harsh conditions to the inhabiting bacterial population in this phyllosphere and their ability to degrade PAH compounds are discussed. Then possibilities of usage of phylloremediation to clean the polluted air from the PAH compounds are discussed.

Keywords: Phylloremediation; Phyllosphere; Polyaromatic hydrocarbons; Bacteria; Bioremediation

Introduction

Polyaromatic hydrocarbons are a group of organic compounds composed of two or more fused aromatic rings of complex organic compounds. Currently, more than 100 different PAHs are available in the environment and out of them 17 PAH compounds were informed as the highly risky compounds by ATSDR [1]. Out of them eight PAHs are typically considered as carcinogenic compounds. These are, benzo(a)anthrocene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, dibenzo(a,h)anthracene, indeno(1,2,3-cd)pyrene and benzo(g,h,i)perylene [2]. But US EPA announced 16 PAHs as priority pollutants. They are, acenaphthene, acenaphthylene, anthracene, benzo(a)anthrocene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(g,h,i)perylene, dibenzo(a,h)anthracene, chrysene, dibenzo(a,h)anthracene, fluoranthene, fluorine, indeno(1,2,3-cd)pyrene, phenanthrene, naphthalene and pyrene. Among them phenanthrene and naphthalene are carcinogenic hazardous compounds which are released in to the air due to vehicular emission and oil refinery processes. The released PAHs into air through these sources come back to earth through the wet and dry deposition methods. Some of these PAHs deposit on plant leaves. Plant leaves are great niches for many microorganisms belong to different groups as bacteria, fungi, algea, protozoa and nematodes. The microbes inhabit plant leaves are called phyllosphere. Bacteria are the predominant microbes in phyllosphere. Deposited PAHs in phyllosphere create a stress conditions to inhabiting bacteria and some of them can adapt to this harsh conditions. As an adaptation they have the capability to degrade PAHs into nontoxic level. This character can be used to remediate the PAH pollutants in the air, water and soil. This bioremediation concept is called phylloremediation.

Polyaromatic Hydrocarbonic Air Pollution

PAH compounds are formed as a result of many anthropogenic sources and some of the natural sources [3]. Natural sources of emissions are prairie and forest fires or volcanic eruptions like natural processes which contribute to the PAHs concentration in the atmosphere. Anthropogenic activities such as coal and oil-fired power plants, automobile and furnace exhausts, gasification/liquefaction of fossil fuels, waste incineration, coke and asphalt production, aluminum smelting and gas, vehicular emission and oil flare operations are sources of these PAH compounds into the air [4]. The results of Muendo et al. [5], convinced the air samples collected from traffic areas of Kenya showed higher PAH concentrations and 30% of collected air samples are consisted with hazardous compounds. According to the results of Edoardo et al. [6], air samples collected from urbanized areas of Europe consisted with highly carcinogenic benzo[*a*]pyrene in the range of 1-20 ng/m^3. Other PAHs, individual concentrations were generally in the range of 1-50 ng/m^3 in Europe. Air pollution of urbanized areas is mostly due to vehicular emission and some oil refinery processes. In fact, main source of napthalenes, phenanthrene, fluoranthene, and pyrene like PAHs are diesel engines [3,7,8]. Other most of PAHs release in to air as a result of vehicular emission and release gasses from oil refinery processes. The research of Nascimbene et al. [9], which was done using lichens as bio monitors around the area of Dolamite in Italy showed PAH concentrations in air are higher along the roadside of this urbanized area other than the rural area far away from this urbanized area. Further, this research revealed phenanthrene, anthracene and pyrene concentrations along roadsides of tested urbanized areas are higher compare to the area away from this road side. Further the results of Blasco et al. [10] indicated phenanthrene is the most abundance PAH pollutant in the air and also other highly prevalence PAHs are fluoranthene, pyrene, naphthalene and benzo(g,h,i)perylene. These 17 PAHs in the environment are highly carcinogenic and genotoxic to the all living beings. Out of these 17 PAHs naphthalene is a highly toxic compound to all the living being. Naphthalene covalently binds to liver, kidney and lung tissue and effect to their function [11]. In addition to that it leads to haemolytic anaemia, nephrotoxicity, dermal and ophthalmological changes. Phenanthrene is also a hazardous PAH which cause photosensitizing of human skin, a mild allergen, potent inhibiting of gap junctional intercellular communication and tumor initiation. Some research indicated the cancer risk of toxicity from acenaphthene, fluranthene and flourene.

Polyaromatic Hydrocarbonic Depositions on Plants

PAHs can be deposited on plants from contaminated soil or atmosphere. This deposition method depends on chemical and physical properties of the pollutant or the environmental condition [12]. But major PAH deposition is from atmosphere. It can be deposited from wet deposition or dry deposition based on the molecular weight of PAH compound. In fact, the lighter, smaller PAHs tend to deposit into plants through dry gasses deposition and the larger, heavier 5 PAHs are usually in particulate form and can be deposited on to plant surface in wet and dry deposition [13]. The deposited amount on the plant leaves depends on PAH concentration of the air and morphological and chemical characteristics of plant leaf [14]. The samples collected from Highway roadsides of Johor showed higher PAH depositions on plants compare to the rural areas. In fact, orange jasmine leaves, Murraya paniculata (L.) collected from roadsides of Bangkok showed 63.99 to 82.46 mg/kg concentration of PAH depositions on the leaves [15]. According to the results of Wang et al. [16], leaf cuticles collected from six tree species from urban Beijing, China, showed 69-mg/kg amount of PAH on leaves. The results of Undugoda et al. [17] revealed the phyllosphere of Ixora chinensis collected from the urbanized areas of Sri Lanka showed the highest naphthalene (160 ng/g), phenanthrene (96.1 ng/g), toluene (85.66 ng/g) and xylene (54.39 ng/g) concentrations. Moreover the results of Bohme et al. [18], revealed the accumulation of PAH on plant leaf surfaces correlate with the PAH concentrations of ambient air. PAHs exist in the air as gasses or particle bound compounds are reached the plant surfaces by both dry and wet deposition [19]. But depositing amount can be vary based on the plant species, morphological and chemical constitutions [20]. The study of Choi et al. [21], revealed, phenanthrene, anthracene, and pyrene concentrations on deciduous forest in Southern Ontario, Canada, were reduced within and above the forest canopy during bud break in early spring.

Bacteria Inhabit the Phyllosphere of Plants

To study planktonic species, water samples were collected once in a month between 7.00 am and 10.00 am for six months (February-July 2017) from all the ten (S1-S10) sampling stations by scooping water at the depth of 15-30 cm below the water surface and filtered through a plankton net with pore size of 64 μm, transferred into 100 ml glass bottles and fixed with acidic Lugol's Iodine solution before transporting to laboratory for identification of species and water analysis. To study planktonic species /diatoms, one drop or 0.2 ml of water from each sample was placed in an Utermohl sampling chamber and kept at least for three hours to get settled [2]. Species were studied using a Zeiss Axioinvert 35 inverted microscope at 40X magnification, identified and confirmed with taxonomic keys [22].

PAH Degrading Bacteria in Phyllosphere

Natural phyllosphere bacteria are able to degrade many air pollutants such as phenanthrene, naphthalene like PAHs and toluene, phenol, ethylbenzene, xylene, like monoaromatic hydrocarbons [22,23]. In fact, microorganisms isolated from the spruce needles showed trichloroacetic acid degradation ability [24]. The results of Charoenchang et al. [25] revealed, the bacterial consortium inhabiting on the dried rain tree leaves have the ability to degrade phenanthrene, fluoranthene and pyrene. The leaf samples collected from four plant species (Ixora chinensis, Evertamia dervaticata, Hibiscus rosa-sinensis and Amaranthus cruentus) which were highly abundant in the urbanized areas of Sri Lanka had phenanthrene and naphthalene degrading bacterial species, Alcaligenes faecalis, *Alcaligenes* sp.11SO, Bacillus cereus, Bacillus methylotrophicus, Serratia marcescens, *Alcaligenes* sp. BC and *Alcaligenes* sp. [26]. The results of Waight et al. [14] revealed, the bacterial species Sphingomonas, Pseudomonas, Microbacterium, *Rhizobium*, and *Deinococcus* spp. which were isolated from six plant species Wrightia religiosa Benth. ex Kurz, Pereskia grandiflora Haw., Hibiscus rosa-sinensis L., Excoecaria cochinchinensis Lour. var. cochinchinensis, *Ixora* sp., and Hymenocallis littoralis Salisb in urbanized areas of Bankok showed higher phenanthrene degradation ability. The results of Ma et al. [27] showed various species belongs to Pseudomonas were able to degrade PAH and the results of Daane et al. [28,29] showed genus Micobacterium had PAH degradation ability. Furthermore, *Rizobium* sp. isolated in the research of Andreoni et al. [30] showed PAH degradation ability. The results of Chang et al. [31] showed the isolated bacterial genus Deinococcus had PAH-degrading activity, this was the only one research which recorded this species for PAH degradation.

Usage of Phyllosphere PAH Degraders for Bioremediation

Over the past decade, the trend of bioremediation has been increased to clean the environment from PAH and other pollutants. The results of Wilson et al. [32], showed many natural soil bacterial species were able to degrade PAH pollutants efficiently. In fact, PAH and phenol-utilizing microorganisms were used to remediate the soil from a creosote plant containing PAHs and phenols using an *ex situ* land treatment process [33]. This showed 82-97% degradation of lower PAHs (and up to 35% degradation of higher PAHs). Bioremediation of oil spills on the shoreline on Delaware Bay was carried out using different types of microorganisms inhabit the soil samples spread around this site [34]. Like these researches initially soil with PAH degrading microorganisms were used to clean the PAH contaminated sites. Then instead of usage of natural soil samples with microorganisms, microorganisms were isolated from the soil and then these isolated bacterial and fungal species were separately added to the contaminated site as bioremediators.

In modern world, PAH degrading bacterial species isolated from contaminated soil are used to clean the PAH contaminated soil sites. In fact, the results of Straube et al. [35] revealed, Pseudomonas aeruginosa strain 64 is able to degrade PAHs in the contaminated soil and sediments. One year after the treatment, 86% of PAH in the site is removed by this inoculum. According to the research of Guerin et al. [33], effective microbes in the compost also can be used to remove PAHs in some contaminated sites. As well as, the results of Sasek et al. [36] revealed PAH contaminated soil exist around a gas plant is remediated using compost inoculum. Moreover, studies of Singleton et al. [37], showed the removal of PAHs present in contaminated soil using the associated bacterial communities who cultured in a bioreactors as semi-continuous or fed batch cultures. Then they showed 76% PAH degrading ability.

Current trend is on use of phyllosphere microorganisms to remediate most of the deposited air pollutants. The results of many modern research revealed, the microorganisms inhabit the phyllophere of plant leaves spread around the contaminated site were able to degrade PAHs deposit on these leaves and these microorganisms can be used as bioremediators. As this we can use phyllosphere PAH degrading bacteria and fungi to remediate the PAH contaminants in the environment this process is called phylloremediation [38-45]. In fact, the results of Forczek et al. [24], revealed, microorganisms colonizing in the spruce needles are responsible for the decrease of trichloroacetic acid in air samples. Moreover, the results of Darlington et al. [22], showed natural phyllosphere bacteria are able to degrade monoaromatic hydrocarbons such as toluene, phenol, ethylbenzene, and xylene. Microbial consortium isolated from dried rain tree leaves in Thailand, were able to degrade phenanthrene, fluoranthene and pyrene very well [25].

The planting ornamental plants with high number of PAH-degrading bacteria in urban areas is a good solution for the environmental PAH pollution. As well as developing biofilters from plant leaves and phyllosphere bacteria for improving indoor air quality. This knowledge is valuable for the development of ecological strategies for minimizing outdoor PAHs and improving air quality in urban areas [46-52].

Conclusions

Phylloremediation is an ecofriendly concept for the air pollution. This concept can easily use for the PAH pollutants which are highly carcinogenic and genotoxic for all the living beings. Phyllosphere of many plants inhabit the polluted areas highly abundant with PAH degrading bacterial and fungal species. Due to these enriched PAH degraders, we can grow these plants with PAH degrading microorganisms indoor and outdoor to remediate pollutants in air after deposition on to these plant leaves. And also by isolating these PAH degrading microbes, biofilters can be introduced to clean the polluted air. All in all phylloremediation is a highly suit bioremediation concept for the air pollution.

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