Project report

On

Differences In Microbes Associated With *Polyalthia longifolia* With Air Pollution

Submitted in Partial Fulfilment of the Requirement for the Degree of
M.Sc. Biochemistry

Submitted by
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CERTIFICATE

This is to Certify that Mr. Deepanshu Rawat has carried out his project work entitled "**Differences in Microbes Associated With *Polyalthia longifolia* With Air Pollution** " under my supervision.

This work is fit for submission for the award of Master Degree in Biochemistry.

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CANDIDATE DECLARATION

I hereby declare that the dissertation entitled "**Differences in Microbes Associated With *Polyalthia longifolia* With Air Pollution**" submitted by me in partial fulfillment for the degree of M.Sc. in Biochemistry to the Division of Biochemistry, School of Basic and Applied Science, Galgotias University, Greater Noida, Uttar Pradesh, It is my original work. It has not been submitted in part or full to this University of any other Universities for the award of degree.

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Deepansu Rawat

List of abbreviation

S. No	Abbreviation	Full form
1	*A. indica*	*Azadirachta indica*
2	CPCB	Central Pollution Control Board
3	CFU	Colony Forming Unit
4	Co_2	Carbon-di-oxide
5	EMB	Eosin Methylene Blue
6	H_2S	Hydrogen sulphide
7	MCA	MacConkey Agar
8	N	Nitrogen
9	NAM	Nutrient Agar Media
10	No_2	Nitrogen-di-oxide
11	P	Phosphorus
12	*P. longifolia*	*Polyalthalia longifolia*
13	P.M	Particulate Matter
14	*P.vulgaris*	*Phaseolus vulgaris*
15	PDA	Potato Dextrose Agar
16	R_2A	Reasoner's 2A Agar
17	S	Sulphur
18	SIM	Sulfide, Indole, Motility
19	So_2	Sulphur-di-oxide
20	VOCs	Volatile Organic Compounds

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ABSTRACT

Trees are well known to remove a significant amount of air pollutants from the atmosphere and should therefore be considered as an integral part of any sustainable plan aimed at improving air quality. The various studies reported that the plants are effective adsorbers of airborne particulate matter. Keeping this in the view, the present study is being carried out on the trees located in polluted area, situated in Noida, Uttar Pradesh. In urban ecosystem of metropolitan city such as Noida constantly exposed to air pollution. Trees alongside roads help in pollution control, therefore, pollution leaves adverse effects to the plants. It interferes with the secondary metabolites of plants and results in permanent damage such as chlorosis, bleaching, necrosis, reduction of leaf thickness. In the present study we try to evaluate the role of ***Polyalthalia longifolia*** associated microbes in high and low levels of particulate matter (pm) pollution in Noida. Alteration in air quality index does not influence plant physiology but also its microbiome.

Also, the present study reported the isolation of colonies of epiphytic and endophytic microorganism present in *Polyalthalia longifolia* grown in polluted area.

However, the study was planned to also isolate the epiphytic microorganism from the same species of plant from less polluted sites. Also, identify both epiphytic and endophytic microorganism. Unfortunately, due to COVID-19 pandemic the abovementioned work was not conducted.

These results may be utilized as sustainable tools for studying plant adaptation to urban ecosystem.

Introduction

Trees are capable of removing a significant amount of air pollutants from the atmosphere and hence should be considered an integral part of any sustainable plan intended at improving air quality. The assumption that plants are important adsorbers of airborne particulate matters is supported by the confirmation obtained from studies dealing with trace elements, pollen, spore, salt, dust, and unspecified particles *(Smith and Staskawicz, 1977)*. Urban forests and trees included in such environment results in increasing quality of air by filtering and uptake of particles and gases *(Beckett et al. 2000)*. Therefore, urban trees are of high importance for the inhabitants, but may also be endangered by exposure to pollution. For example, in leaves of a typical urban roadside tree species, *Platanus orientalis* air pollution caused changes in chlorophyll content and peroxidase activities *(Alaimo et al. 2000; Baycu et al. 2006)*. Pollutants results in damaging stomata, injury of leaf and early senescence, also reduce activity of photosynthesis, permeability of membrane and decreases growth and some sort of plant yield. *(Tiwari et al.2006)*. Secondary pollutant such as ozone(0_3) results in affecting plants shows severely ranging injuries i.e visible to higher susceptibility for pathogens and resulting in reduction of plant productivity *(Krupa et al., 2000; Greggy et al., 2003; Karlson et al., Ainsworth et al., 2012)*. Results in decreasing productivity of plant. When ozone goes through leaves reaching to stomata give reacting oxygen species and leades to oxidative stress, results in decrease of activity of photosynthesis, growth of plant and accumulation of plant *(Ainsworth et al., 2012)*. Oxides of nitrogen such as (NOx) were found to affect plants that depends on concentration, exposure length, species, stage of plant development and site characteristics that leads to visible injury such as wilting, necrosis or even defoliation *(Taylor et al., 1975)*.When Dust deposited on surface of leaf, consisting of ultrafine and coarse particles, results in decreasing plant its effect through *(Bender et al., 2002)* process of exchange of leaf gas.*(Ernst, 1982)*. Decreasing production of leaf results in decreasing area of leaf and number of leaf increased senescence *(Seyyednejad et al., 2011)*. Deposition of dust on leaf causes occlude stomata *(Hirano et al., 1995)*. Opening of stomata cause enter to Particle affects their level of toxicity, activities of plant *(Farmer, 1993)*.Leaf that shows enhanced acidic nature that gives type of air pollutants such as named Sulphur dioxides (SO_2), NOx that leads to diffuse and leads to form acid radicals in leaf matrix when reacts with water content of cell that affects leaf chlorophyll *(Turk and Wirth, 1975)*. Rate of transpiration during process of photosynthesis affected by pollution. It has been reported that permeability of cell that increases pollutants in air

(Keller, 1986). Chlorophyll pigment causes stress resulting in exist to organized state goes under several photochemical reactions such as reversible bleaching, oxidation, and reduction *(Puckett et al., 1973).*

Extensive communication occurs between plants and microorganisms during different stages of plant development in which signaling molecules from two partners play an important role *(Bais HP et al., Park Sw et al., Weir TL et al., 2004).* Diverse mechanisms are involved in plant-microbe interaction *(Whipps 2001; Compant et al., 2005).* Plant and microbial interaction, under many type of individual cases few mechanisms are required *(Berg et al, 2002; Haas and Defago 2005; Miller et al., 2009).* Microbial activity improved by three process that helps in improving plant health 1. Controlling the hormones signaling in plants *(Verbon and Liberman 2016).* **2.** Revolting microbial strains of pathogens *(Mendes et al, 2013)* **3.** Rising bio-accessibility of native nutrients in soil *(Van der Heijden et al. 2008)*. Bacteria associated with plant releases some hormones which helps in hormonal balance of plant. Hormone balance importance can be shown by Ethylene. Some plant species like *Arabidoposis thaliana* can be promoted at lower level while this is known as senescence hormone which inhibits plant growth *(Pierik et al., 2006)*. Nutrients like Phosphorus, Sulphur, and Nitrogen in the ecosystem joined in organic molecules having less bio-accessibility for plants. To acquire such nutrients plants depends for growth on soil microbes like fungi and bacteria which have metabolic process to break polymer and contains organic forms of Phosphorus(P), Sullphur (S) and Nitrogen(N). Microbial contents in cell are mostly get free either through turnover and cell of cell, or by protozoic depredation *(Bonkowski 2004; Richardson et al., 2009).*

Review of Literature

Ashoka *(Polyalthia longifolia)*

Table-1 **Classification**

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Magnoliales
Family	Annonaceae
Genus	*Polyalthia*
Species	*Polyalthia longifolia*

Figure- 1 *Polyalthia longifolia* (Ashoka)

Polyalthia longifolia commonly known as Ashoka, is native to India. It is an evergreen tree with the height of 6 m to15m *(Katkar et al., 2010). Ashoka* is tolerant to air pollution and is effective in alleviating noise pollution as well. Trees have capacity to absorb pollutants like P.M, CO_2, oxides of sulphur and nitrogen *(Kapoor et al., 2013).* Hence it is recommended for 'green belts' for the mitigation of air and noise pollution *(Kapoor et al., 2013).*

Overview of Noida pollution status

Noida is one of the many megacities struggling with rapid urbanization and gigantic levels of pollution from industrial, residential and transportation sources. Change in land use, urbanization and population growth has affected ecosystem of Noida leading to increase in air pollution which adversely affects the living organisms dependent on it. Harmful substances and gases such as particulate matter, especially PM_{10} and $PM_{2.5}$, Sulfur dioxide (SO_2), Nitrogen oxides (NO_2), ground level ozone, volatile organic compounds (VOCs) and carbon

monoxide are polluting the air. Construction work and automobiles are widely acknowledged to have been important sources of air pollution in Noida, with the transportation share growing rapidly in recent years.

Effect of pollutants on trees

Mostly two types of injuries can be caused in plants like Acute and Chronic injury. More often unveiling to a massive accumulation of gas for a brief time leads to acute injury and shows few detectable symptoms on parts of plants like necrotic bruise so these injuries become easy to notice. Most tangible injury such as chronic injury caused low gas aggregation under prolonged unveiling and shows growth form and reductions in yield, not showing visible symptoms to analyze pollution effect in plant life like different aspects complete development and growth.*(Gupta and Ghouse, 1987; Misra and Behera, 1994)*, foliar morphology *(Farooq et al. 2000; Pal et al. 2000; Shrivastava and Joshi, 2002; Gostin, 2009; Sukumaran, 2012)*, anatomy *(Garg et al. 2000)*, and biochemical changes *(Garty et al. 2001; Mashitha and Pise, 2001; Gavali et al. 2002; Rai et al. 2013; Rai et al. 2013; Rai and Singh, 2015; Rai, 2016,).* Aggregation and Integration of pollutants in plants take place which being exposed in environment. It is reported on the basis of their toxicity level, plants have many biochemical change and certain change in metabolites take place *(Agbaire and Esiefarienrhe, 2009)*. Plants Effect being exposed to results of pollution causes long term changes that occur in plants *(Joshi et al. 2009)*. Many studies have been done on the basis of plants health observation exposed to automobile and other particulates suspended in air on various physiological and morphological parameter in many plants studies by many workers *(Naidoo and Chirkoot, 2004, Verma and Singh, 2006; Prajapati and Tripathi, 2008a; Rai et al. 2013; Rai and Panda 2014; Rai and Singh, 2015; Rai, 2016,).*

Morphological impact of PM on plants

Plant having primary receptors such as leaves for pollutants like gaseous and PM of atmosphere. Configuration of plant leaves changed by pollutants as they interact with leaf outer surface area and leads to modification. Polluted area containing deposition of cement on pine and spruce, number of density of needle scars whereas reduced in less polluted area.*(Ots et al. 2011)*

Demonstration shows dust of cement being treated plants such as *Brassica campestris*(Mustard) shows biomass reduction in plants affects activity of photosynthesis, regulate reduction in growth, oil content and yield control over plants *(Shukla et al. 1990)*

Physiological and biochemical effect on plants or plant leaves-

Plants development and growth aspects is affected by airborne particulates depends on their chemical and physical nature. Dust leads to change leaf pH extract. Stomata sensitivity changed by air pollutants due to change in leaf extract of PH. Calcium hydroxide liberated by hydration of leaf surface by dust cement deposited which raises leaf surface alkalinity at pH 12. Wax components and lipid hydrolyzed by alkalinity, leaf cuticle being penetrated and caused to degrade protein. *(Guderian, 1986; Czaja 1960,).*

Relative water content

Plant life has biggest importance of water. Plant regulates uniform release and uptake of water *(Jones, 1994)*. Under stress condition increased water content helps the plants *(Singh and Verma, 2007)*. High relative water content favors resistance in plants *(Dedio, 1975)*. Total leaf surface area mainly affected by plant having sufficient water content *(Schuppler et al. 1998)*.

Table-2. Effects of pollutants on plant.

PM Pollutants Effects	**References**
Acute injury: yellow leaf spots. Leaf rolling, interveinal	*Taylor et al.,1986*
necrosis, inhibition of leaf expansion. Leaf curling Taylor et al.,1986 Blocks the stomata of leaf	*Pierce, 1909*
Cause cell plasmolysis, inhibit starch production which ultimately leads to cell death	*Czaja, 1961*
Cell destruction, bark peeling, leaf necrosis	*Czaja, 1961*
Increase in leaf spotting fungus	Taylor et al.,1986
Increase transpiration	Duggar & cooley, 1994
Increased water loss	Eveling, 1969
Inhibit pollen germination	Anderson, 1914
Reduce catalase activity	Borka, 1980
Reduce photosynthesis and increase leaf necrosis	Darley, 1966
Reduced seed set	Ramgasamy & Jambulingan,1973
Reduced vegetative and reproductive growth, reduced tissue nitrogen, calcium increased phosphorous	Singh & Rao, 1978

Pigment content, photosynthesis, and stomata

Photosynthetic pigment such as chlorophyll, responsible for productivity found in chloroplast in leaf. This is also called Photoreceptor which is damaged by air pollution. Air pollution causes degradation in photosynthetic pigment observed by many workers *(Prusty et al., 2005).* This was observed by a number of workers *(Bansal, 1988; Singh et al. 1990; Sandelius et al. 1995).*

Chlorophyll existence occurs in extremely organized state and exposed to stress causing many biochemical reaction such as oxidation, reduction, and reversible bleaching *(Puckett et al. 1973)*

hence Plant physiological and morphological behavior depends on any change in chlorophyll concentration. Leaf having depleted content of carotein and chlorophyll *(Chauhan and Joshi 2008)*. Photosynthesis physiological aspects can be studied by analyzing properties of chlorophyll a (Chl a) in photosystem II (PSII) *(Govindjee et al. 2004; Bussotti et al. 2010),* and has been used in plant investigations of stress(Maxwell and Johnson, 2000; *Adams and DemmigAdams, 2004; Bussotti et al. 2010. Bussotti et al. 2010)* analyzed ozone impact in stress condition in woddy plant alongwith with chlorophyll a fluoroscence. Black dust responsible for gaseous exchange and less photosynthetic activity in leaves of *Viburnum tinus (Thompson et al. 1984)*. Further, limestone dust deposition on leaf causes decrease in overall plant performance through loss many harmful effects to plants caused by deposited dust of limestone on leaf surface which causes reduction in plant performance by decreasing electron transport closing chlorophyll content, oxygen complex being uncoupled, inhibiting assimilation of CO_2 of chlorophyll content, in Namib Desert shrub, *Zygophyllum prismatocarpum* (Tall zygophyllum) *(van Heerden et al. 2007)*.

Dust deposited on leaves results in changing normal shape of stomata to occlude. *(Hirano et al. 1995)* because of entering of particulates through opening of stomata and toxicity *(Farmer, 1993)* like photosynthetic rate, plant growth inhibition, rate of photosynthesis *(Armbrust, 1986),* late flower and imbalance of hormones *(Farooqui et al. 1995)*. Translocation caused by inhibiting of net photosynthesis resulting in decreased leaf area.

Apart from PM, *(Mansfield and Majernik 1970)* CO_2 and SO_2 impact being shown in their concise review except PM on behavior of stomata.*(Black and Unsworth 1980)* observed and described the *Phaseolus vulgaris* (Kidney Bean), fumigation of SO_2 causes to open stomata. However, In general, closing of stomata mainly caused by pollutants to a certain threshold concentration *(Freer-Smith and Taylor, 1992)* which causes to direct damage of stomata *(Krajickova and Mejstrik (1984)* observed that in P. vulgaris (Kidney Bean), and Zea mays (corn), power plant emmiting fly ash diffusion of gases in stomata.

Sugar content

Living organisms needs necessary source of energy in form of soluble sugar. Process like photosynthesis and respiration needs sugar *(Tripathi and Gautam, 2007).* In difficult situation more consumption of sugar caused by Pollutants like SO_2, NO_2 and H_2S. Inhibition of photosynthesis and stimulated respiration rate mostly similar to reduced in total content of sugar *(Tzvetkova and Kolarov, 1996).* Similarly, *(Bucker and Ballach 1992)* soluble carbohydrates level tends to decrease by many mixture fumigation such as O_3, SO_2 and NO_2 both in young and mature leaves. Under stress condition leads to high metabolic rate tends to decrease soluble sugar *(Lorenc-Plucinska, 1982).* A higher inhibition of photosynthesis and photorespiration in the less resistant individuals were established in *Pinus Sylvestris* (Scotch pine) by *(LorencPlucinska 1982).*

Protein

All enzymatic machinery performed by important component such as protein in plant species. Under stress condition depends on plant species resistance component such as protein helps in protecting plant from pollution. Pollution leads to degrade protein *(Prasad and Inamdar, 1990). (Constantinidou and Kozlowski, 1979)* Protein content reduction mainly caused by increased protein denaturation or breakdown of previous protein to amino acids *(Kumar and Dubey, 1998)* have also concluded that pollutants coming out of autoexhaust may cause inhibitory effect on protein synthesis. Pollutants leads to change in protein *(Iqbal et al. 2000; Singh and Jothi, 1999).* Photosynthetic activity reduced because of disturbed protein *(Constantinidou and Kozlowski, 1979*; *Singh et al. 1988). Rana et al. (2000)* observed concentrations of SO_2 on the quantity of free proline in the anthers of *Brassica juncea* L (mustard). Pollutants on plants include pigment destruction, depletion of cellular lipids and peroxidation of polyunsaturated fatty acid *(Tiwari et al. 2006).*

Effect of pollutants on Trees associated microbes

Phyllosphere is generally a hostile environment because of constant fluctuations in temperature relative humidity and sunlight exposure. Accordingly, only selective microbial communities withstand such a habitat *(Lindow and Brandl, 2003).* Being present at the interface of plant surface and the atmosphere, these microorganisms are also known to affect plant volatile emission, and in turn, these volatiles may have a significant role in determining the microbial composition in phyllosphere *(FarréArmengol et al. 2016).* Change in air quality causes degradation of biomass *(Fenn et al. 1989; Phillips et al., 2002; Karnosky et al. 2005).* Moreover, some of the pollutants can be used by phyllosphere bacteria as a carbon source *(Sandhu et al. 2007).*

Microbial composition on Phylloplane

The composition of microbes present in phyllosphere differs from species to species *(Whipps et al. 2008).* A study of 56 tree species reported that each species harbors distinct microbial communities in phyllosphere *(Redford et al. 2010).* These microbial communities are generally dominated by Proteobacteria, such as *Methylobacterium* and *Sphingomonas*, *Beijerinckia*, *Azotobacter*, *Klebsiella*, and Cyanobacteria such as *Nostoc, Scytonema, and Stigonema* also reside in the phyllosphere *(Vacher et al. 2016).* Population of γ-Proteobacteria such as Pseudomonas could be high as well *(Delmotte et al. 2009; Fierer et al., 2011; Bodenhausen et al. 2013; Kembel et al. 2014).* Predominant fungal species reported in phyllosphere of (plant name/s) was Ascomycota, of which the most common genera are *Aureobasidium, Cladosporium, and Taphrina (Coince et al. 2013; Kembel and Mueller 2014).* Furthermore, yeasts belonging to genera *Cryptoccoccus and Sporobolomyces* was found abundant in phyllosphere of (plant name) *(Cordier et al. 2012; Ottesen et al. 2013).* Studies on *Azadirachta indica* have reported microbes belonging to Actinomycete such as *Streptosporangium sp*., *Microbispora sp*, Aureus, Greseofuscus, Albosporus, Cinereus, Globisporus, Lavendulae, *Nocardia sp*. and fungus such as *Eupenicillium parvum, Phomopsis oblonga, Cladosporium cladosporioides, Pestalotiopsis sp., Trichoderma sp, Aspergillus sp. (verma et al. 2009; Kusari et al. 2012).* In tree species *Magnifera indica*, the species of microbes such as *Pseudomonas sp., Alcaligenes sp.*, and *Micrococcus* has been reported *(Ilori*

et al. 2006). A study on *Prosopis juliflora* (Algaroba mesquite) has reported 32 bacterial species *(Mazinani et al. 2017).*

These studies clearly show the adverse effect of air pollution on trees. They also provided the information about microbial association with trees and air pollution, as well as the effect of pollution on the phyllospheric microbial ecosystem. Thus, in view of the above the present study aims to provide an insight on the changes occurring on tree ecosystem with respect to its associated microflora.

Aims and Objectives

1. Identification of Less polluted and more polluted locations in Noida region-

- Location with significant high and low PM2 and PM10 levels will be identified according to Central Pollution Control Board (CPCB).
- From the above list 2 locations in Noida will be shortlisted for polluted and non-polluted areas.

2. To identify type of tree species around polluted and non-polluted area of Noida-

- On each shortlisted location, number and type of tree species will be identified using quadrate method.

Materials and Methods

Description of Polluted Sites

Noida sec-1

The latitude and longitude of 28.5884° N, 77.4270° E it is located near to sec -16. It is very busy place, roads are stuck mostly with traffic. Centre of pollution and control board is also located here. As this is an industrial hub so much crowd is there.

The present study has been carried out in Noida sec 1

Fig-2. Noida Sector-1 (Source Google Map-2020)

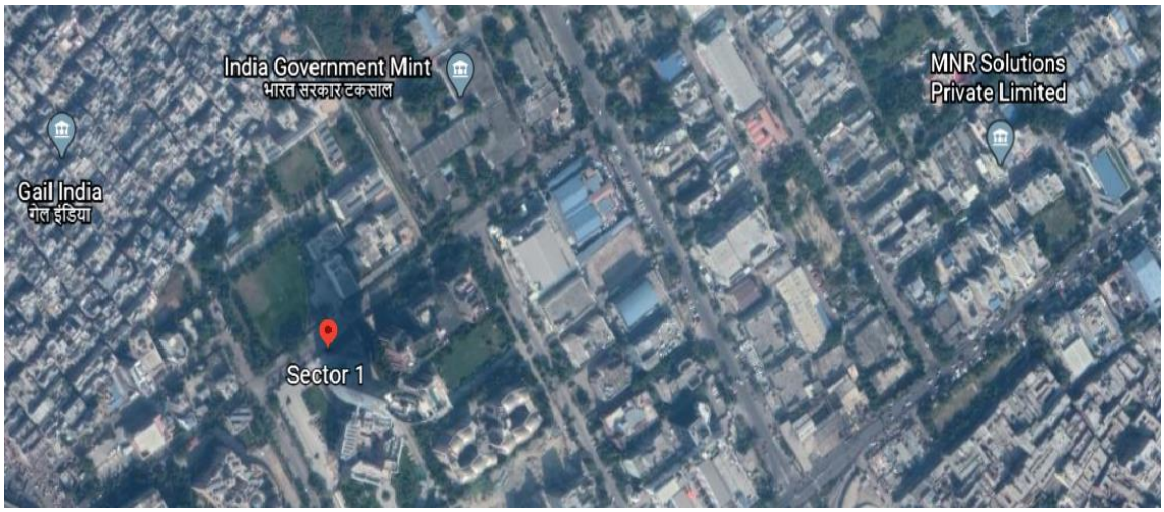


Fig-3 Noida Sector-1 (Source Google Earth View -2020)

Fig-4 Roadside view Noida-sec 1 (Source Google Earth View -2020)

5a

5b

5c

5d

Fig- 5 (a-d) Sample collection site

Less Polluted sites

Due to covid-19 pandemic and lockdown, sample from less polluted site could not be collected.

Description of Less Polluted Sites- Not visited yet because of covid-19 epidemic

Media Preparation

Nutrient Agar Media (NAM): it is a general purpose, nutrient medium used for the cultivation of microbes supporting growth of a wide range of non-fastidious organisms. Nutrient agar is popular because it can grow a variety of types of bacteria and fungi, and contains many nutrients needed for the bacterial growth.

Ingredients	Gms / litre
Tryptone	1.8
Sodium chloride	1.8
Yeast extract	0.9
Agar	2.7
Final pH (at 25°C)	7.4±0.2

Procedure:

Suspended 2.7 gram (agar) in 180 ml distilled water. Add tryptone-1.8gm, Nacl-1.8gm, Yeast extract-0.9gm to it. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. If desired, the medium can be enriched with 5-10% blood or other biological fluids. Mix well and pour into sterile Petri plates.

Potato Dextrose Agar (PDA): it is used for the cultivation of fungi. Potato Dextrose Agar is a general-purpose medium for yeasts and molds that can be supplemented with acid or antibiotics to inhibit bacterial growth. It is recommended for plate count methods for foods, dairy products, and testing cosmetics. PDA can be used for growing clinically significant yeast and molds. The nutritionally rich base (potato infusion) encourages mold sporulation and pigment production in some dermatophytes.

Ingredients	Gms / litre
P.D Broth	7.2
Agar	4.5
Final pH (at 25°C)	5.6±0.2

Procedure:

Suspended 7.2 gram of P.D Broth in 120 ml distilled water, add 4.5gm agar to it. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well before dispensing. In specific work, when pH 3.5 is required, acidify

the medium with sterile 10% tartaric acid / lactic acid. The amount of acid required for 100 ml of sterile, cooled medium is approximately 1 ml. Do not heat the medium after addition of the acid.

Reasoner's 2A agar (R2A): Agar was developed by Reasoner and Geldreich for bacteriological plate counts. A low nutrient medium, such as R2A Agar, in combination with a lower incubation temperature and longer incubation time stimulates the growth of stressed and chlorine-tolerant bacteria. Nutritionally rich media, such as Plate Count Agar (Standard Methods Agar), support the growth of fast-growing bacteria but may suppress slow growing or stressed bacteria. When compared with nutritionally rich media, R2A Agar has been reported to improve the recovery of stressed and chlorine-tolerant bacteria. R2A Agar is recommended in standard methods for pour plate, spread plate and membrane filter methods for heterotrophic plate counts.

Ingredients	**mg / litre**
Casein Enzymatic Hydrolysate	30
Peptic digest of animal tissue	30
Casein Acid Hydrolysate	60
Yeast extraxt	60
Glucose	60
Starch	60
Dipotassium Phosphate	3.6
Magnesium Sulphate	60
Agar	1.8 gm
Final pH (at 25^0c)	7.2

Suspended 1.8g (agar) in 120 ml distilled water. Add the entire chemical as weighed to it. maintain the PH. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°CMix well and pour into sterile Petri plates.

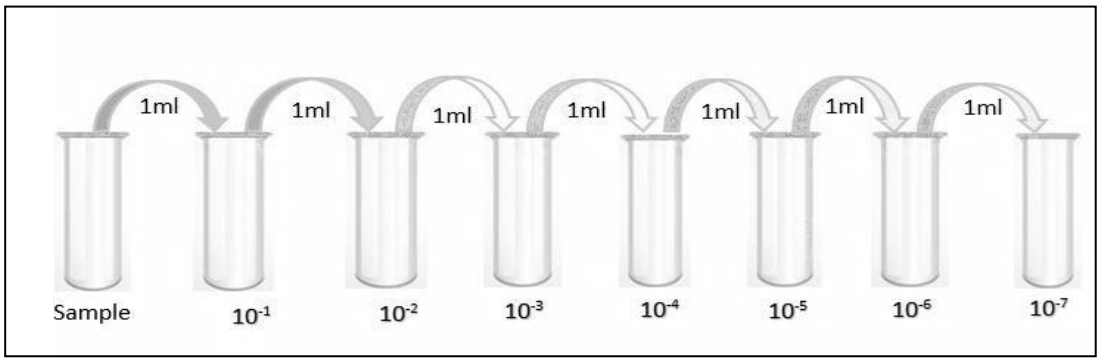
Identify type of tree species around polluted and non-polluted sites: Quadrat method (Gleason, 1920; Mahajan, & Fatima, 2017) is used for the identification of the type of tree species present at all four sites.

1. Using meter tape on each site three quadrat of 200m X 200m was selected randomly.
2. Visually differentiate types of tree species.
3. Take the picture of each species by camera.
4. On each shortlisted location, type of plants species will be identified according to quadrate method.
5. Noted and counted the type of tree species.
6. Tree species selected based on common trees in all four sites and have known a pollution decreasing effect
7. One common tree species is selected for study.

Isolation of leaf associated microbes:

1. Collection of leaves from each species was dine in the plastic zip lock and sealed tightly.
2. Pre-autoclaved all vessels and equipment and sterilize under the laminar flow by the help of Ultra violate light just before using for half hour.
3. Five leaf of each tree was collected and prewashing of the leaf material was done with distilled water for few seconds to remove dust and other pollutants. The endophytes were isolated as described by *Mareque et al. (2015)*.
4. Under laminar flow washed leaf was macerate in mortar pastel in a 0.9% NaCl (crushing buffer). This leaf macerate was collected for the isolation of leaf endophytes.

5. Serial dilution of each leaf macerate was done as per fi. For the first dilution, 1ml of macerated leaf sample was pipetted and was added to 9ml of double distilled water (DDW) in a test tube and was labeled as 10-1. This process was repeated up to dilution10-7.



6. Isolation of microbes was done using Nutrient Agar Media (NAM) for fast growing bacteria, Potato Dextrose Agar (PDA) for fungi, Reasoner's 2A agar (R2A) for slow growing bacteria *(Kiyohara et al., 1982). (Larone et al., 1987) (Reasoner and Geldreich, 1985)*

7. Washes and dilutions was inoculated on the agar media plate. For NAM wash 2, wash 4, wash 5 and 10^{-5}, 10^{-6}, 10^{-7} dilutions were used. For PDA 10^{-3}, 10^{-4}, 10^{-5}, wash 2 was used and R2A 10^{-4}, 10^{-5}, 10^{-6}, wash 2 And incubate Petri-plates at 28^{0}C temperature for 24-48 hours (NAM), 48hours to 7 days (PDA), 24hours to 7 days (R2A).

8. Post incubation, the plates were observed for respective microbial Colony Forming Unit (CFU) to estimate the total number of microbial load on the tree leaf using the following formula

$$\text{CFU/ml} = \frac{\text{(total number of colonies on plate X dilution factor)}}{\text{Volume of culture plate}}$$

The observations for the colony morphology was made using the following key:

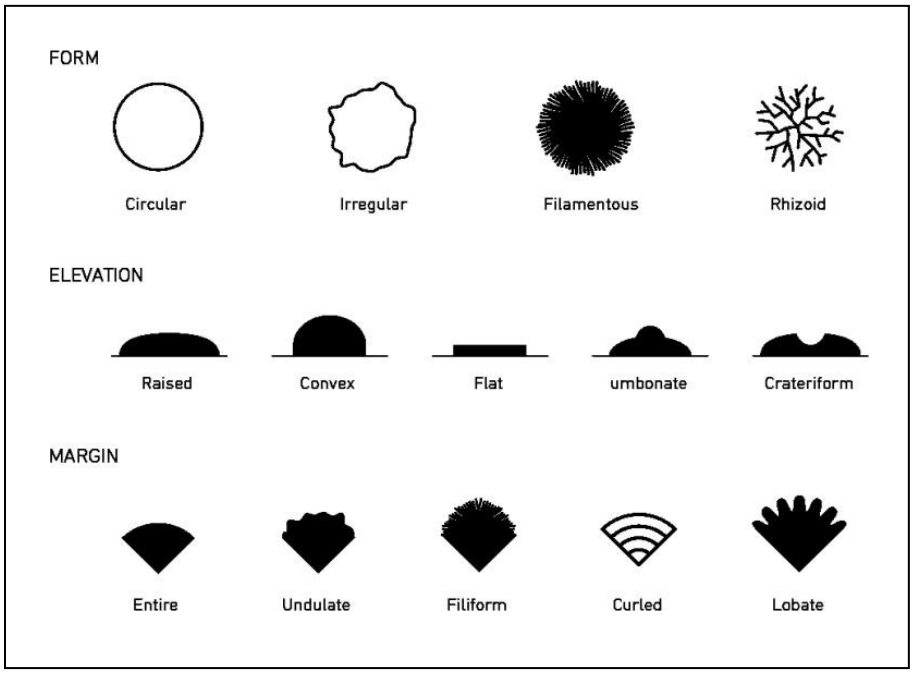


Figure 6. Morphology of microbial colony.

Biochemical characterization of microbes was done by Eosin Methylene Blue (EMB) Agar, MacConkey Agar (MCA), SIM (Sulfide, Indole, Motility) Medium, Simmons Citrate Agar (SCA), Lysine Decarboxylase Test (LDT), Catalase Test.

Eosin Methylene Blue (EMB) agar

a) Pre prepared Eosin Methylene Blue (EMB) (Himedia, Cat no.: M317-100G) was used in sterilized disposable Petri plates (Abdos®).

b) Using purified colonies from an 18-24-hour culture on solid media, NAM, loop full culture was inoculated on the Eosin Methylene Blue (EMB) by streaking plate culture method.

c) Incubate the inoculated medium aerobically at 35ºC for 18-24 hours.

d) Colonies with purple to pink color as positive test for gram negative bacteria and no color for gram positive bacteria were observed.

MacConkey Agar (MCA)

a) Pre prepared MacConkey Agar (SRL, Cat no. 76875 (MM 011) media was used in sterilized disposable Petri plates (Abdos®).

b) Using purified colonies from an 18-24 hour culture on NAM, NAM, loop full culture was inoculated on the MacConkey Agar Medium by streaking plate culture method.

c) Incubate the inoculated medium aerobically at 35ºC for 18-24 hours.

d) Colonies with red to pink color as positive test for gram negative, lactose fermenting bacteria and colorless/ transparent for gram positive, non-lactose fermenting bacteria were observed.

SIM (Sulfide, Indole, Motility) Medium

a) Pre prepared SIM (Himedia, Cat no.:M 181-500G) Medium was used as slants in test tubes.

b) Using purified colonies from an 18-24-hour culture on NAM, NAM, loop full culture was inoculated the SIM Medium by stabbing the center of the medium to a depth of 1/2 inch.

c) Incubate the inoculated medium aerobically at 35ºC for 18-24 hours. The colony formed away from the stab, were recorded as motile colonies.

d) H_2S three drops of Kovacs Reagent (Himedia, Cat no.: R008-100G) was added to the surface of the of the colonies. The colonies that gave effervescent were recorded as H_2S positive.

e) Positive Indole test was observed as pink to red colonies upon

Simmons Citrate Agar (SCA)

Method of use:

a) Pre prepared Simmons Citrate Agar (SCA) media (Himedia, Cat no.: M099-100G) was used as slants in test tubes.

b) Using purified colonies from an 18-24-hour culture on NAM, loop full culture was inoculated on the Simmons Citrate Agar (SCA) Medium by streaking plate culture.

c) Color change from green to blue was recorded as positive result.

Lysine Decarboxylase Test

Method of use:

a) Pre prepared Lysine Decarboxylase (Himedia, Cat no.: M376-100G) Medium was used for test tube slants.
b) Using purified colonies from an 18-24 hours culture on NAM, loop full culture was, inoculated the Lysine Decarboxylase media tubes.
c) Incubate the inoculated medium aerobically at 35ºC for 18-24 hours.
d) Positive test was observed as Purple or yellow color.

Catalase Test-

Method of Test

a) Use a loop or sterile wooden stick to transfer a small amount of colony growth in the surface of a clean, dry glass slide.
b) Few drops of 3% H2O2 (SD, Cat no.:20276 L05) were placed on the glass slide.
c) Presence of oxygen bubbles was recorded as catalase positive.

Results

Ashoka *(Polyalthia longifolia)*- CFU/ml in Ashoka as well no. of individual colonies in *P. longifolia* was high in polluted area. The no. of epiphytic colonies were more than the endophytic colonies in this area. However, the total no. of unique microbes associated with the tree (based on morphology) was higher in polluted areas.

The specific bacterial colony were named according to their morphological appearance by giving them a specific numbering or named by certain coding. It makes easy to recognize the same colony on again repetition.

Table 3: Trees observed in polluted site (Noida sector 1)

Common Name	Scientific Name
Arjun	*Terminalia arjuna*
Ashoka	*Polyalthia longifolia*
Bargad	*Ficus benghalensis*
Ber	*Ziziphus jujube*
Chamrod	*Ehretia laevis*
Frangipani	*Plumeria rubra*
Jamun	*Syzygium cumini*
Kaner	*Nerium indicum*
Karanj	*Pongamia pinnata*
Krishna fig	*Ficus benghalensis var. krishnae*
Neem	*Azadirachta indica*
Pipal	*Ficus religiosa*
Saptaparni	*Alstonia scholaris*
Toot	*Morus alba*

Air Quality Index-

Table-4 Air Quality Index

Pollutants	Prescribed Standard	Concentration of Noida sec 1	Concentration of Puss Hill Forest (Less polluted)
PM <2.5	60 µg/M^3	128 µg/M^3	71 µg/M^3
PM <10	100 µg/M^3	427 µg/M^3	91 µg/M^3
Ozone	180 µg/M^3	49 µg/M^3	95 µg/M^3
Nitrogen Dioxide	80 µg/M^3	105 µg/M^3	32 µg/M^3
Sulphur Dioxide	80 µg/M^3	11.9 µg/M^3	24 µg/M^3
Carbon Monoxide	04 Mg/M^3	1.82 Mg/M^3	0 µg/M^3

Polluted Site Pollution Data-

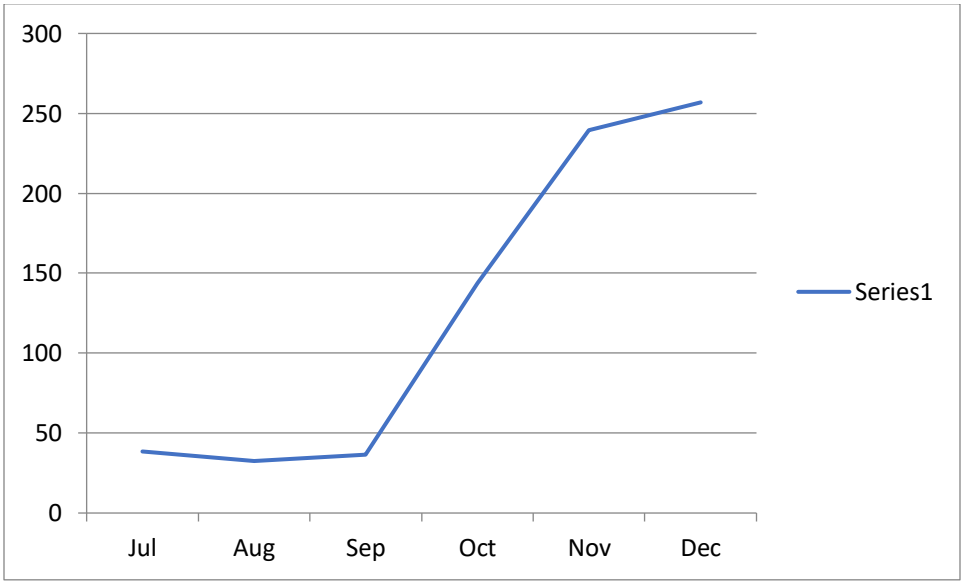


Figure 6: Six months average data of PM-2.5 (Noida sec-1, 2019)

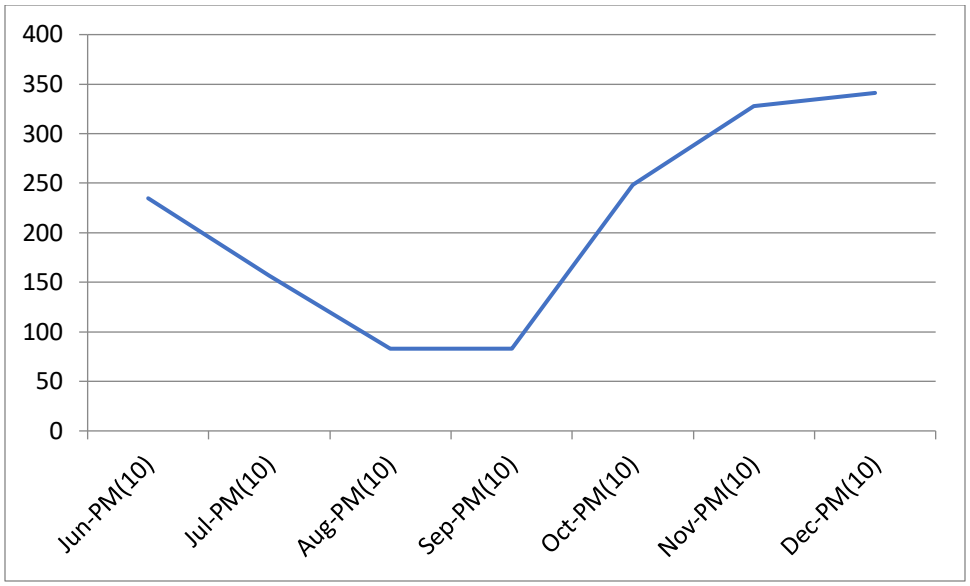


Figure 7: Seven months average data of PM-10 (Noida sec-1, 2019)

Less Polluted Site Pollution Data-

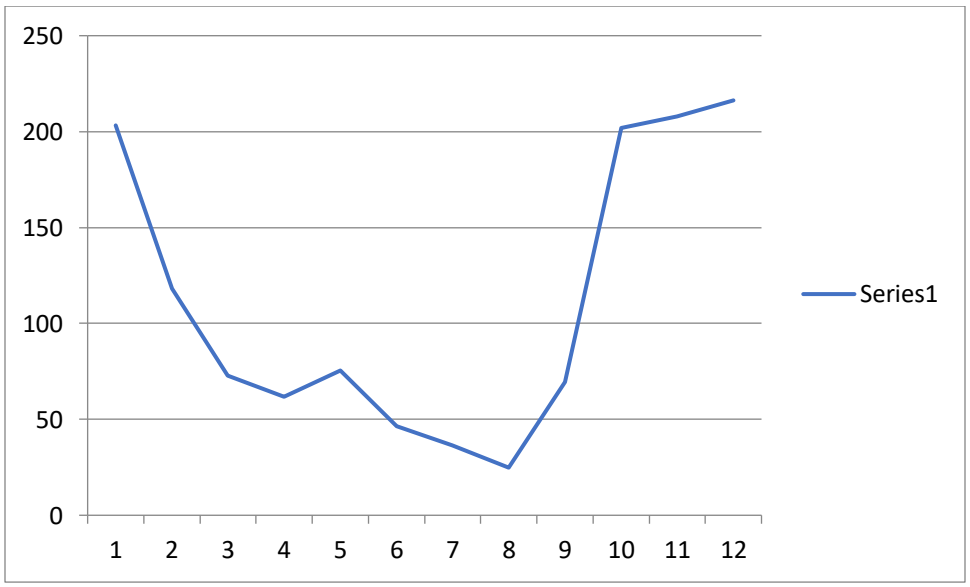


Figure 8: One year average data of PM- 2.5(Noida sec-1, 2019)

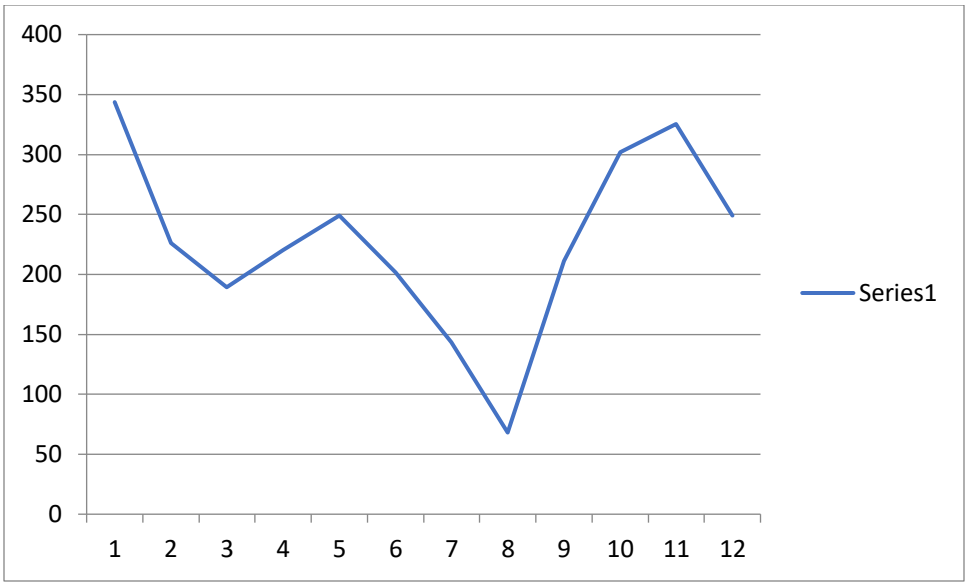


Figure 9: One-year average data of PM- 10 (Noida sec-1, 2019)

Microbial Characterization based on their appearance:

The microbes being characterized based on their appearance along with the help of chart. Observing bacterial colony tells the characteristics.

The observations for the colony morphology was made using the following key:

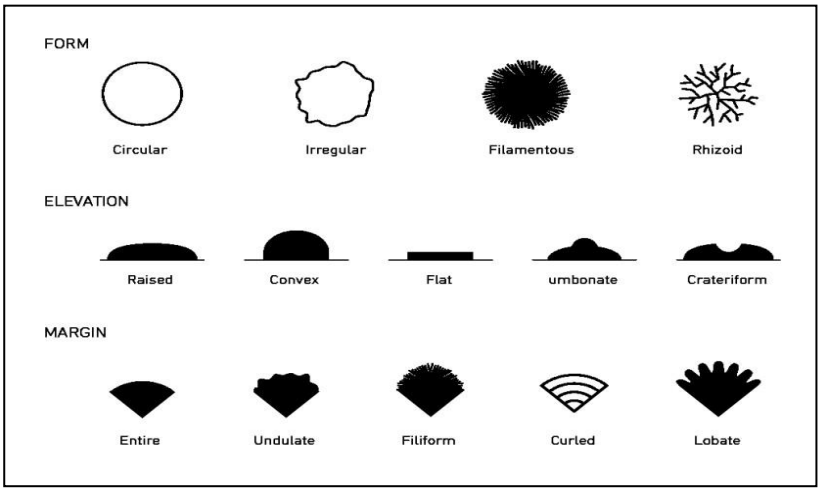


Table3: Colonial morphology of endophytic microorganism

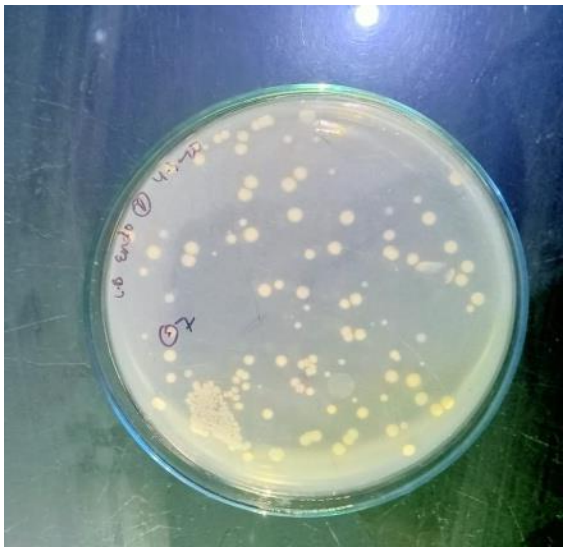
	L.B					
	Endophytes					
Dilution factor	**Form**	**Shape**	**Elevation**	**Margin**	**Colour**	**Dry/sticky**
10^{-4}	Round	Small	Raised	Entire	White	Dry
10^{-5}	Round	Small	Raised	Entire	White	Dry
10^{-6}	Round	Medium	Raised	Entire	White	Dry

Table3: Colonial morphology of epiphytic microorganism

Epiphytes					
Form	**Shape**	**Elevation**	**Margin**	**Colour**	**Dry/sticky**
Round	Small	Raised	Entire	White	Dry

7a

7b



7c 7d

Figure 7(a-d): Colony count of epiphytic microorganism- a: wash-2(used for leaf wash); b-d: colonies observed in 10^4, 10^5, 10^6 dilution factors.

Table 4: Colony count of microorganism of obtained from *Polyalthia longifolia.*

Locations		**Ashoka (*Polyalthia longifolia*)**		
		CFU /ml	No. of different types of colonies	
Noida sector-1	**Epiphyte**	3.8 X 10^4	14	Total 18*
	Endophyte	3.0 X 10^6	8	

Conclusions

The present study revealed high occurrence of both epiphytic (3.8×10^4) and endophytic (3.0×10^6) microorganisms originated from *P. longifolia* collected from the polluted site showed high number and different type (n=18) microorganism. Unfortunately, the study did not reveal the characterization of isolated microorganism. Also, due to lockdown circumstances the present study could present the comparison data with less polluted site which was the plan of study.

Pending Work

- Due to lockdown less polluted site visit could not be possible.
- Biochemical tests are left.
- Microbial analysis of polluted site sample left.

Future Scope

Microbes help plants in many physiological functions. My study shows that pollutants do not only affect humans but also trees and the microbes associated with them. Thus, the prospects of my study are as follows:

- Future studies may focus on the effects of the changes in the microbial population on the physiology of the trees.

- Use of plant associated microbes as bio indicator: Presence of certain microbes may indicate the abundance of the specific pollutant.

- Bio- remediation: Presence of a microbial species in areas with high quantity of a certain pollutants, also indicates that the microbe has adapted itself for that pollutant. Therefore, these microbial species can be used for utilization of the pollutant and hence, used for remediation.

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